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## CONTENTS

### No. 1 (1959)

GOTOH, Kanji: Intra-Inbred Line Variation in Number of Tassel Branches of Corn.....	1
TSUCHIYA, Takumi: Genetic Studies in Trisomic Barley. I. Relationship between Trisomics and Genetic Linkage Groups of Barley.....	14
YOSHIDA, Tadao: Life-Cycle of a Species of <i>Batrachospermum</i> Found in Northern Kyushu, Japan .....	29
SHARMA, Arun Kumar and BHATTACHARYYA, Nripendra Kumar: Chromosome Studies on Four Different Species of <i>Cinnamomum</i> .....	43
FUKASAWA, Hirotsuke: Nucleus Substitution and Restoration by Means of Successive Backcrosses in Wheat and its Related Genus <i>Aegilops</i> .....	55
ARASAKI, Seibun and SHIHIRA, Ikuko: Variability of Morphological Structure and Mode of Reproduction in <i>Enteromorpha linza</i> .....	92
CHOWDHURY, N. P.: Observations on the Structure and Ecology of a Xerophytic <i>Selaginella</i> from India. 1. <i>Selaginella bryopteris</i> (L.) Baker.....	101
IWAKI, Hideo: Ecological Studies on Interspecific Competition in a Plant Community. I. An Analysis of Growth of Competing Plants in Mixed Stands of Buckwheat and Green Grams .....	120

### No. 2 (1960)

NAKAJIMA, Goichi: Cytogenetical Studies on the Intergeneric F <sub>1</sub> Hybrids between <i>Triticum macha</i> and Four Species of <i>Secale</i> . .....	139
SHARMA, A. K. and BHATTACHARYYA, N. K.: An Investigation on the Scope of a Number of Pre-Treatment Chemicals for Chromosome Studies in Different Groups of Plants .....	152
SUTO, Tiharu and SUGIYAMA, Shintaro: Sex Expression and Determination in Spinach. I. Growth Habit and its Sex-limited Inheritance .....	163
TSUCHIYA, Takumi: Cytogenetic Studies of Trisomics in Barley.....	177
HOTTA, Yasuo: The Role of Protein and Ribonucleic Acid in the Differentiation of Fern Gametophyte .....	214
SHIBATA, Mannen and ISHIKURA, Nariyuki: Paper Chromatographic Survey of Anthocyanin in Tulip-Flowers, I .....	230
TAZAKI, Tadayoshi: On the Growth of Pine Yearlings in Coastal Dune Regions with Special Reference to Their Drought Resistance .....	239
HOGETSU, K., OSHIMA, Y., MIDORIKAWA, B., TEZUKA, Y., SAKAMOTO, M., MOTOTANI, I., and KIMURA, M.: Growth Analytical Studies on the Artificial Communities of <i>Helianthus tuberosus</i> with Different Densities .....	278



## No. 3 (1961)

KUSUMOTO, Tsukasa: An Ecological Analysis of the Distribution of Broad-Leaved Evergreen Trees, Based on the Dry Matter Production .....	307
TSUDA, Michio: Studies on the Halophilic Characters of the Strand Dune Plants and of the Halophytes in Japan .....	332
TEZUKA, Yasuhiko: Development of Vegetation in Relation to Soil Formation in the Volcanic Island of Oshima, Izu, Japan .....	371
TAKEDA, Tomoshiro: Studies on the Photosynthesis and Production of Dry Matter in the Community of Rice Plants .....	403
SHARMA, A. K. and BHATTACHARYYA, U. C.: Colchicine Effect on Pollen Mother Cells and Pollen Grains of <i>Zebrina pendula</i> Schnizl .....	438



## INTRA-INBRED LINE VARIATION IN NUMBER OF TASSEL BRANCHES OF CORN<sup>1)</sup>

KANJI GOTOH<sup>2)</sup>

Problems connecting with variance due to inbred lines have been pursued by the various ways. Recently interesting questions have been derived from the results of comparison between variances due to inbred lines and those due to their  $F_1$  hybrids. Although the results in such comparison are not always consistent, it has been sometimes pointed out that inbred lines are usually susceptible to environmental variations than  $F_1$  hybrids.

In the case analysing quantitative inheritance, variance due to parental varieties is, in general, assumed as the estimate of environmental variances, and differences in variance have been sometimes found between parental varieties. Further we have been forced to transform the data to logarithm etc. in such cases. We have, however, few biological informations for the variance due to inbred lines.

In relation to these problems, it might be of interest to know the actual situation for the magnitudes of variability in long inbred lines. To ascertain the magnitudes of intra-inbred line variation in corn, the author conducted this experiment.

Data were taken at the Agronomy Farm of Iowa State College in Ames, Iowa, in 1956 and 1957. The long inbred lines and the double haploid lines dealt with have been maintained so far in the Iowa Agr. Exp. Sta. The author wishes to express hearty thanks to Dr. G. F. Sprague for his kindness and generosity permitting of usage of his valuable materials and giving facilities for the experiment.

### MATERIALS AND METHODS

Whole materials used in this experiment were grown with no replications for the other purposes, mainly, for the propagation. It has precluded direct analysis of the data. Thus it was necessary to check the variance due to replications, using another designs of experiment. Almost of the long inbred lines were consisted of two to three sib-lines. Then the differences between sib-lines were tested.

Detailed designs of each experiment will be shown later in each section. Character dealt with is number of the tassel branches. It seems likely that in this character the pressures of artificial selection might be weaker than those in the economic characters. Number of the tassel branches was counted in each individual except the main branch.

In the text, LIL stands for the long inbred line or lines. Except one line

1) Contributions from the Tokachi Branch Station of Hokkaido Agr. Exp. Sta., Obihiro, Hokkaido, Japan.

2) Tokachi Branch of Hokkaido Agricultural Experiment Station, Obihiro, Japan.

which was  $F_6$  generation in 1956, whole inbred lines belonged to the category of the long inbred line. Further, diploid lines which were induced by colchicine treatment with haploid plants were designated as double haploid lines. They will be called as DHL in the text.

In 1956, these materials suffered from severe drought in summer, and in some inbred lines it was difficult to get enough seeds. On the other hand, the plant growth in 1957 was vigorous and until the period when the data were taken, it seemed to be normal year.

## EXPERIMENTAL RESULTS

### 1. Variance due to replications

As mentioned before, the inbred lines were grown with no replications. Consequently, one might doubt about the magnitudes of variances due to replications. To make clear this point, two designs of experiment with replications were analysed.

#### a) In the case of 5 $F_1$ hybrids (in 1956)

5  $F_1$  hybrid populations were grown under the randomized block arrangement with 5 replications. Average number of the tassel branches in each  $F_1$  hybrid was as follows: WF9×Oh43, 18.9; AD×C103, 23.9; 67×C103, 19.4; WF9×B6, 19.3; AD×67, 18.9, respectively.

TABLE 1

Analysis of variance due to replications in 5  $F_1$  (left) and in  $F_3$  (right)

Variation (in 1956)	d. f.	m. s.	Variation (in 1957)	d. f.	m. s.
Bet. $F_1$ hybrids	4	22.95**	Bet. lines	56	77.87**
Bet. reps.	4	2.71*	Bet. reps.	2	1.60
Error	16	0.76	Error	112	0.53

Table 1 (left) shows the result of analysis of variance. The difference between  $F_1$  hybrids was highly significant. Although variance due to replications was significant at the 5% level, its magnitude was negligible, comparing those of variance due to  $F_1$  hybrids.

#### b) In the case of $F_3$ lines (in 1957)

57  $F_3$  lines derived from the cross between Oh45 and B14 were examined. They were grown under the randomized block arrangement with 3 replications. 20 plants were chosen at random for counting the tassel branch numbers. Average number of all  $F_3$  lines was 9.12, ranging from 5.8 to 14.2. The result of analysis of variance is shown in the right of Table 1.

Variance due to  $F_3$  lines was significant at the 1% level, however, those due to replications was non-significant and negligible.

From the results of two designs of experiment we might be able to assume



that the variance due to replications, was, in general, very small in our experimental field.

## 2. Variance due to sib-lines (in 1957)

Almost of the LIL used in 1956 consisted of a pair of sib-lines, and two to three sib-lines of a line were arranged in the succeeding rows in 1957. Can one deal with the average of such sib-lines as the representative value of a line? Of all lines examined in 1957, 22 and 19 lines were consisted of three and two sib-lines, respectively. To ascertain the differences between sib-lines, analysis of variance was conducted.

TABLE 2  
Analysis of variance due to sib-lines

Variation (22 lines)	d. f.	m. s.	Variation (19 lines)	d. f.	m. s.
Bet. lines	21	111.85**	Bet. lines	18	103.13**
Bet. sib-lines	2	1.16	Bet. sib-lines	1	0.05
Error	42	1.28	Error	18	1.12

In both cases the differences between lines were significant at the 1% level, whereas variances due to sib-lines were smaller than error variances. The magnitude of differences between means of the sib-lines was various, line by line. If these differences were due to the genetic factors, they might be ascribable to the polygenic segregations. Based on these results, means and standard deviations of each line were calculated, averaging means and standard deviations of the sib-lines.

## 3. Coefficient of variability of inbred lines (in 1956)

In 1956, 20 LIL and 11 DHL were examined. The former consisted of a pair of sib-lines and the latter had one independent line for almost of the lines. Table 3 shows the mean and coefficient of variability (*c. v.*) in each line.

As seen from the table, averages of means and *c. v.* in LIL were 18.2 and 15.0%, and those in DHL were 16.9 and 14.2%, respectively. Then the degree of *c. v.* in LIL ranged from 9% to 31%, whereas its range was rather narrow in DHL, namely, from 6% to 20%.

Correlation coefficient between means and standard deviations in 31 lines was 0.5721. From this figure one might be able to assume that means and variances are not independent in the present materials. Then the data were transformed to logarithm, and using the same procedure, correlation coefficient was calculated. Thus minus signed correlation coefficient, namely,  $-0.6842$ , was obtained. According to this result, the data obtained in 1957 were not transformed also.

## 4. Coefficient of variability of inbred lines (in 1957)

In 1957, 42 LIL and 12 DHL were dealt with. Of 42 lines 22 had 3 sib-lines, 19 had 2 sib-lines and last one was independent line. In DHL, 10 lines had 2



TABLE 3  
Mean and coefficient of variability in 1956

LIL			DHL		
No. of plot	Mean	<i>c. v.</i> (%)	No. of plot	Mean	<i>c. v.</i> (%)
1001	15.4	10	1197	12.4	12
1009	26.0	14	1198	18.1	14
1011	22.1	12	1199	11.4	20
1033	14.5	16	1200	12.7	19
1035	25.0	10	1240	16.4	14
1046	12.1	14	1241	23.2	16
1048	8.6	23	1243	26.2	6
1057	14.5	19	1244	17.2	16
1067	16.1	11	1268	11.1	16
1077	11.5	25	1290	21.6	9
1079	18.4	16	1315	15.6	14
1081	27.1	13	On the average	16.9	14.2
1083	18.9	13			
1089	29.9	10			
1091	37.9	9			
1093	7.9	31			
1107	15.1	10			
1131	17.9	12			
1169	13.4	12			
1181	11.7	19			
On the average	18.2	15.0			

sib-lines and two lines were independent one. Table 4 shows means and *c. v.* of the examined lines.

The degree of *c. v.* ranged from 13% to 48% in LIL, whereas the range for DHL was rather narrow, from 15% to 34%. As seen from the table, wide range of variation was found in *c. v.* among DHL. Theoretically, phenotypic variance due to DHL should be real environmental variance. However, it is quite probable that difference may exist between lines in phenotypic variance in usual field conditions.

##### 5. Comparison of coefficient of variability in both years

11 LIL and 4 DHL were examined in both seasons. The data for these lines were summarized in Table 5.

Means of each line in 1957 were lower than those in 1956, whereas *c. v.* (23.1% on the average) in 1957 were higher than those (15.5% on the average) in 1956. As mentioned earlier, in the season of 1956 the drought condition affected seriously to plant growth. However, number of the tassel branches in each line was uniform among the individual plants, while in 1957 variance of the

TABLE 4  
Mean and coefficient of variability in 1957

LIL			No. of plot	Mean	c. v. (%)
No. of plot	Mean	c. v. (%)			
			1129	6.7	43
1001	9.1	23	1132	8.2	24
1004	16.6	21	1141	10.9	17
1007	17.1	21	1145	5.8	48
1017	16.1	25	1149	26.3	14
1019	10.5	18	1151	17.0	18
1026	9.1	34	1159	13.5	16
1034	10.8	28	1166	12.1	20
1037	7.1	25	1175	7.7	27
1040	12.6	27	1177	16.8	25
1046	16.4	16	1184	12.4	16
1048	17.5	22	1900	37.3	14
1053	8.6	28	1906	11.2	37
1055	12.9	14	On the average	13.9	23.1
1058	18.0	13	DHL		
1060	14.2	20	1186	11.6	19
1063	16.0	16	1190	14.2	26
1065	18.8	20	1191	12.8	17
1068	19.0	26	1193	13.7	31
1071	18.7	17	1207	17.3	16
1074	7.9	32	1209	13.8	27
1080	6.1	34	1213	19.4	16
1086	23.8	21	1217	15.9	17
1089	4.4	33	1225	16.6	26
1092	5.9	29	1227	24.2	15
1095	27.1	14	1260	5.9	34
1103	12.1	18	1270	15.2	19
1109	12.2	21	On the average	15.0	21.9
1121	13.2	18			
1124	16.2	17			

inbred lines was relatively high, although, they say, it was normal year. In the Table 5, W17R-B had the lowest mean, and its *c. v.* was highest in both years. On the other hand, HD 2189 showed the lowest *c. v.* in both years. Correlation coefficient between the *c. v.* of 1956 and those of 1957 was very high, namely, 0.7097. This result shows that each inbred line has its inherent magnitude of *c. v.* in our condition.

#### 6. Extension of the analysis

##### a) Coefficient of variability of representative inbred lines (in 1957)



TABLE 5

Comparison of mean and coefficient of variability in 1956 and 1957

Name of lines	Mean		<i>c. v.</i> (%)	
	1956	1957	1956	1957
B-2	15.4	9.1	10	23
B-35	14.5	10.8	16	28
B-38	12.1	12.6	14	27
I-198	14.5	8.6	19	28
Hy	16.1	18.8	11	20
W22R-1	27.1	23.8	13	22
W17R-B	7.9	4.4	31	33
A 265	15.1	12.1	10	19
Oh07	17.9	16.2	12	17
CI 28A	11.7	7.7	19	26
HD 502	11.4	14.2	20	26
HD 698	12.7	12.8	19	17
HD1795	11.1	13.8	16	27
HD2189	21.6	24.2	9	15
41-2504B×MO-572	18.2	16.7	14	19
On the average	15.2	13.7	15.5	23.1

Based on the same manner described above, the *c. v.* of the 5 inbred lines was calculated (Table 6). 100 plants were examined in each line. Their *c. v.* was 20.4% on the average. It was a general tendency among the 5 populations that their modes showed higher value than their means.

*b) Coefficient of variability of  $F_1$  hybrids*

One might be interested in the comparison of environmental variation of parental varieties and their  $F_1$  hybrids. In the 5  $F_1$  hybrids dealt with in Section 1, averaged *c. v.* was 12.5%. This value was lower than those obtained from LIL and DHL, namely, 15.0% and 14.2%, respectively (see Table 3).

Similar result was obtained in an experiment regarding analysis of the quantitative inheritance of the tassel branch numbers. Results are shown in Table 7.

TABLE 6

Performance of 5 inbred lines in 1957

Name of line	Mean	$s^2$	<i>c. v.</i> (%)
Hy	19.58	7.6198	14
WF 9	14.42	8.6905	20
187-2	11.91	6.0221	21
I-205	14.34	5.5196	16
B-37	5.08	2.5390	31



TABLE 7  
Statistics in parents and  $F_1$  hybrids

Parent and $F_1$	Mean	Standard deviation	<i>c. v.</i> (%)	$F_1$ /Mid-parent	$F_1$ /Large-parent
B 14	10.1	1.22	12.1		
K 148	16.1	3.10	18.7		
GG 208	18.9	2.68	14.2		
B 38	13.3	1.73	13.0		
B 14 × K 148	15.8	2.02	12.8	121	98
B 14 × GG 208	18.7	2.57	13.7	129	99
B 38 × K 148	19.0	3.25	17.1	129	118
GG 208 × B 38	21.1	1.54	7.3	131	112
K 148 × GG 208	29.1	3.72	12.8	166	154

TABLE 8  
Degree of heritability in broad sense

Cross	No. of $F_2$ plants	Mean	$s^2$	Heritability (%)
B 14 × K 148	89	15.1	13.43	62.3
B 14 × GG 208	91	15.3	9.81	48.1
B 38 × K 148	99	17.2	19.01	57.7
GG 208 × B 38	90	21.1	10.27	59.3
K 148 × GG 208	90	24.2	29.74	65.7

Averaged *c. v.* of 4 varieties was 14.5%, whereas those of 5  $F_1$  hybrids among them was 12.7%. Although data are scarce, and standard deviation of hybrids itself is sometimes larger than those of their parents, it seems likely that *c. v.* of  $F_1$  hybrids is smaller than those of their parents in this character.

*c) Heterosis and heritability in broad sense*

Based on the above mentioned data the degree of heterosis was calculated (see Table 7). Although the degree was fairly different between the crosses, heterosis in this character was remarkable.

$F_2$  hybrid populations of the same crosses were used for calculation of heritability in broad sense. Table 8 shows the results of estimation. The degree of heritability was 58.6% on the average of the 5 cross combinations. This value was unexpectedly high, however, an unpublished data obtained from a replicated trial suggested that heritability calculated by means of variance components basing on line means may be higher than those shown above.

## DISCUSSION

According to the analysis of experimental data, it was shown that the phenotypic variation of LIL and DHL was unexpectedly remarkable, and the degree of *c. v.* varied among the examined lines. The degree of *c. v.* of 15 inbred lines examined in both years was correlated each other ( $r=0.7097$ ). Then it was assumed that phenotypic variation of inbred lines might be a kind of inherent character within a certain environment. As shown before, correlation coefficients between means and standard deviations of the inbred lines were 0.5721 in 1956 and 0.6910 in 1957, and the result of log. transformation of the data in 1956 was not effective (Log. transformation of data has been recommended in such cases where undesirable correlation found between means and variances in the analysis of quantitative inheritance). Further, correlation between means and *c. v.* was calculated, and correlation coefficient was  $-0.6376$  in 1956 and  $-0.6248$  in 1957, respectively. This means that the degree of *c. v.* shows inverse proportion to the mean. Next model will explain the situation briefly.

Mean	(i)		(ii)		(iii)	
	<i>s</i>	<i>c. v.</i>	<i>s</i>	<i>c. v.</i>	<i>s</i>	<i>c. v.</i>
100	4	4	4	4	8	8
50	3	6	2	4	3	3
25	2	8	1	4	1	1

In all three cases, correlation coefficient between means and standard deviations (*s*) will show positive values. Only in the case of (i) one may have negative correlation between means and *c. v.*

Gotoh [7] found remarkable variability in the response to the critical sowing date in an old local strain of barley variety. Connecting with this finding and results obtained from a series of experiments, he discussed the relation between potential variability of cereal varieties and their differentiation. It is easy to imagine that some of the inbred lines of corn might have the genetic variations, heterozygosity or heterogeneity, like as the above mentioned cereal strain. In other words, variability in invisible or quantitative characters of inbred lines may superimpose the magnitudes supposed by us (see Jones [10]). Bailey et al. [1] pointed out that sub-lines of two inbred strains of house mouse had diverged spontaneously at the certain rate.

In our experiment 1 to 3 lines were grown per line, from one of which 1 to 3 plants were selected and these sib-lines succeeded a line further. Consequently, it is surmised that the differences between sib-lines may be due to heterozygosity, or polygenic segregation. According to Table 2 (left and right), the differences between sib-lines were non-significant and negligible. On the other hand, Hooker [8] found heterogeneity (within inbred line) in the 5 long inbred lines of corn in

the resistance of seeding to *Pythium*. The situation may be easily explained as these inbred lines were derived from the mixtures of the considerable numbers of sub-lines.

Mutation is one of the important factors inducing diversification of inbred lines. In nature there are strains or genotypes with high mutability. Genes increasing mutation rate, or genes stimulating mutations of near locus have become familiar in our field [3, 9, 12]. As has been pointed out by Schmalhausen [14], certain mutation might induce lines with high mutability, and such high mutability may be a genetic character. Thus it is plausible that among our long inbred lines with the considerable magnitudes of variability some ones may be highly mutable. Here the author have, however, no evidences. Ross et al. [13] discussed along the same line. They presented the cases showing stability and unstability of really homozygous lines. As well known, in *Nicotiana* East [6] found the increased variation in the succeeding generations, whereas Lindstrom [11] was not able to find any variation in later generations in the tomato. They stated further, "If such a difference in mutability may be found between species, it is possible that a similar difference in propensity to variability may be found between strains within the one species."

Neglecting the genetic sources of variation, let assume that the variance of LIL or DHL is mostly due to the environmental variation. We could divide their environmental variance into following components;

- (1) a part due to experimental error.
- (2) a part due to environmental variation itself, resulting from the mechanical accidents, irregularity of fertilizer level and so on,
- (3) lastly, a part due to self-regulating ability (meaning of which will be described in detail later) of plants themselves, in other words, resistance of plants to environmental fluctuations, and this ability may control magnitudes of developmental error, phenotypically.

First and second parts should arise as the results of chance fluctuations, and any plants examined in certain environments are always forced to suffer from such fluctuations. Third part has a biological meaning. In order to discuss this point further, it may be appropriate to present an example. It was obtained from an experiment concerning fertilizer levels in rice. The experiment was conducted by Dr. H. Oka in Formosa. Based on standard deviations of his data, following figures were calculated. The author wishes to express his thanks for his kindness offering the valuable data.

Actually, 4 levels of fertilizer were used in the experiment. However, we will concern only 3 levels, namely, no fertilizer (0), standard (I), and double of standard (II). Date of heading will be dealt with here, and Table 9 shows the results of calculation. Varieties, Taichung No. 65 and 150, belong to the *Japonica* group of rice, and the others to the *Indica* group. In the Table, meaning of each symbol is as follows:

$\bar{V}$ =variance of the standard plot on the average of four blocks

$\bar{V}t$ =averaged variance of three treatments on the average of four blocks

$Vvt$ =variance of variances in three treatments



TABLE 9

Statistics obtained from experiment of fertilizer levels in rice varieties

Variety	Date of heading			$\bar{V}$	$\bar{V}t$	$Vvt$
	0	I	II			
1st crop						
Taichung No. 65	May 25	May 23	May 25	5.98	8.45	15.59
Taichung No. 150	May 19	May 17	May 19	13.23	15.12	28.21
U-cheng	June 5	June 3	June 3	6.51	6.20	1.61
Peh-ku	May 23	May 22	May 22	5.01	6.02	2.18
Peh-mi-fen	May 27	May 26	May 27	6.60	8.62	11.97
2nd crop						
Taichung No. 65	Sept. 27	Sept. 26	Sept. 27	6.67	8.61	7.31
Taichung No. 150	Sept. 23	Sept. 23	Sept. 24	2.54	3.33	2.10
Song-chiang	Oct. 5	Oct. 1	Oct. 1	1.77	2.09	0.49
Koh-tu	Oct. 4	Oct. 3	Oct. 2	2.13	1.93	0.38
Peh-mi-fen	Oct. 3	Oct. 2	Oct. 2	8.56	8.97	14.07

As seen from the table, the magnitude of variances of Taichung No. 150 was different between the first and the second crop. In the second crop the variance of this variety reduced remarkably. Following is the distinct points in the climatic conditions between two seasons; long day and low temperature in the first crop, and short day and high temperature in the second crop. Consequently, it may be assumed that day length, temperature and difference between day and night temperature would affect strongly the magnitudes of environmental variations of varieties. The magnitudes of  $\bar{V}$  and  $\bar{V}t$  were parallel. So it may be not necessary to mention further. Then remarkable difference was found between varieties in the magnitudes of  $Vvt$ . This value shows how environmental variance of the varieties varied by the treatments of fertilizer. It is noticeable that  $Vvt$  of the *Indica* type of varieties except Peh-mi-fen was very small. It means that these varieties are not sensitive to the fertilizer levels. In our sense, *Indica* type has usually strong self-regulating ability for the amounts of fertilizer. Now, we could presume that certain varieties or inbred lines have their own nature for the self-regulation, and that environmental variance of varieties or lines may be controlled, more or less, by their ability of self-regulation. If it is true, we may be able to suppose that between varieties or lines of crop plants are there differences in the self-regulating ability. Thus, there should be some lines which show always relatively small variances in spite of the changes in environmental conditions.

Depending on the differences of years, locations, and cultural conditions etc., the relative magnitudes of environmental variance of LIL or DHL may increase or decrease. However, a mode of the magnitude could be settled, like as a focus of certain lens. In particular point of environmental populations, certain lines

should show the smallest environmental variance, and the further the point where the lines were moved, the larger their environmental variance.

As mentioned previously, the inbred lines examined showed higher means and smaller magnitudes of variance in 1956 than those in 1957. We had the dry season in 1956 and so-called normal year in 1957. So far it has been difficult to explain such situation from the biological point of view. Following citation may help some for further understanding of the problem. Becker [2] reported about the uncontrolled phenotypic variability of the rat body weight. According to him, "starvation reduced the mean and raised the variability and the animals grown under the better maternal environment of  $F_1$  dams were less variable than those rats littered and raised by the inbred dams." Then he suggested that "the slower the growth the greater the variability within a group of organisms."

Clayton, Morris, and Robertson [4] presented an idea separating the environmental variance into two parts, i.e. variance due to "true environmental error" and variance due to "developmental error." The latter derived from the variance of difference in bristle numbers between 4th and 5th sternites of *Drosophila melanogaster*. Furthermore, according to Clayton and Robertson [5], the difference was found between lines in the rate of variance due to developmental error ( $\sigma_D^2$ ) and total phenotypic variance ( $\sigma_P^2$ ), namely,  $\sigma_D^2/\sigma_P^2$ . The rate ranged from 0.19% to 0.78%.

In the above mentioned case, developmental error was dealt with as the results of irregularities in the expression of genes "within individuals". Now, let extend this category to "between individuals." Theoretically, homozygous lines should have no variations between individuals. However, during the processes of development genic expression of individuals could be modified in various ways. In such case, self-regulating ability will affect the degree of modification in genic expression.

According to our experiment, it has been explored that there are differences between LIL or DHL in environmental variance. We can replace the LIL or DHL with the pedigrees in the cross-breeding. In the early generations of cross bred progenies, phenotypic variance is mostly attributable to genic sources. However, in the later generations, there should be lines with the low self-regulating ability among the lines with large variances, in other words, the lines with low self-regulating ability might accompany the considerable magnitudes of environmental variances. In the case of selection in plant breeding above mentioned situation should be taken into account. Besides, a few of lines with large variance might have high mutability.

In the analysis of quantitative inheritance, we are used to estimate the environmental variance depending upon the averaged variance of whole materials concerned. From the results of our experiment, it is plausible that the environmental variance of the pedigree lines includes the variance due to self-regulating ability of lines themselves, besides "true environmental variance" and variance due to "experimental error", which are common through the whole lines concerned.

## SUMMARY

1. The variation in number of the tassel branches of long inbred lines (LIL) and double haploid lines (DHL) of corn were analysed, based on the data taken in two years. As the results of experiment, it was shown that the phenotypic variation of LIL and DHL was unexpectedly remarkable and the degree of *c. v.* varied among the examined lines, namely, from 9% to 31% in LIL and from 6% to 20% in DHL in 1956, and from 13% to 48% in LIL and 15% to 34% in DHL in 1957, respectively.

2. The degree of *c. v.* of 15 inbred lines examined in both years was highly correlated each other ( $r=0.7097$ ). Thus it may be concluded that phenotypic variation of inbred lines is an inherent characteristic of them.

3. It was found that coefficient of variation of the  $F_1$  hybrids is smaller than those of their parental varieties and that some  $F_1$  hybrids show remarkable heterosis in this character. The degree of heritability of this character was 58.6% in broad sense on the average of 5 cross combinations.

4. Correlation coefficients between means and standard deviations of inbred lines were 0.5721 in 1956 and 0.6910 in 1957. However, log. transformation was not effective.

5. In relation to the variations in the magnitudes of phenotypic or environmental variance between inbred lines detailed discussions were presented. Variance due to inbred lines were separated into three parts, i.e. 1) due to experimental error, 2) due to environmental variation itself, and 3) due to self-regulating ability of plants themselves. Then it was surmised that self-regulating ability should be different between lines, and that there will be some lines which show relatively small environmental variances in spite of the environmental changes.

6. Lastly, it was suggested that among the pedigree lines derived from crossing in plant breeding there should be lines with low self-regulating ability which might accompany large environmental variances.

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# GENETIC STUDIES IN TRISOMIC BARLEY, I.

## RELATIONSHIPS BETWEEN TRISOMICS AND GENETIC LINKAGE GROUPS OF BARLEY<sup>1), 2)</sup>

TAKUMI TSUCHIYA<sup>3)</sup>

Although all the seven possible linkage groups in barley were thought to be established by means of ordinary genetic analyses [18, 19, 20, 22] (Robertson, Wiebe and Immer [18], Robertson, Wiebe and Shands [19, 20], Smith [22] and others), cytogenetic studies made by Kramer, Veyl and Hanson [13] and Burnham [3] have revealed the fact that the two linkage groups, III and VII, are associated with one and the same chromosome. Further the independence of other 5 linkage groups and the interrelationships between each chromosome and respective genetic linkage groups of barley have been almost established by extensive cytogenetic studies of reciprocal translocations [1, 3, 8, 9, 10, 13]. However, some points have not yet been decisively ascertained; the relationships between chromosome 5 and linkage group II and chromosome 7 and linkage group V are remained to be determined [3, 4, 7].

Since the present author was successful in inducing all of the seven possible types of primary simple trisomics [25], an attempt was made to approach the same problem with the use of these trisomics. This paper describes the results of genetic studies in these barley trisomics, together with the brief accounts of their characteristics.

## DESCRIPTION OF THE PRINCIPAL CHARACTERISTICS OF TRISOMIC BARLEYS

All the seven possible types of the primary simple trisomics were induced by the present author [25, 29] from autotriploid plants of *Hordeum spontaneum* C. Koch var. *transcaspicum* Vav., a wild two-rowed barley [24, 27]. In sharp contrast to the spontaneous barley trisomics found by Kattermann [12] and Smith [21] and the trisomics from heterozygotes of the X-ray induced reciprocal translocations [15], all of the trisomics produced by the present author have high fertility whenever they are selfed or crossed with normal diploids. This made it possible to utilize the trisomics very effectively for the genetic analyses.

Since a detailed description of the characteristics of the primary trisomic types will be given in another paper [29], only the principal morphological as well as cytological features are described below.

1) Abstract of the content dealt with in this paper was read at the 28th Annual Meetings of the Genetics Society of Japan, held at Toyama, Japan, Oct. 6-8, 1956. (*Jap. Jour. Genet.*, 31: 313-314)

2) The present studies were partly supported by grants for the Scientific Research Fund from the Ministry of Education.

3) Kihara Institute for Biological Research, Mutsukawa, Minami-ku, Yokohama, Japan.

### 1. *Bush*

This plant type has the most distinctive, abnormal characters among the trisomics described herein. The name Bush comes from its dwarf growth with abundant tillers. Its leaves are very narrow and short, the leaf index (length/width)<sup>1)</sup> being larger (20.0) than in the normal diploid (16.4). The first and second foliage leaves of Bush are smaller and of darker green color, which distinguishes readily this trisomic type from the normal diploids at the earliest stage of growth. Frequent occurrence of the onion-like fused leaves also facilitates its identification. Multiple or compound spikelets at some of the rachis nodes are rather common in Bush; this character may be said to be one of the most remarkable qualitative characters among the many diagnostic and distinctive traits of all trisomic types. Degeneration of the anthers at various stages of their development is not infrequent; in 185 out of 565 spikelets examined, or 32.7 percent of the spikelets had one or two degenerated anthers in each. Nevertheless, normally developed anthers contained as much as 97.2 percent of good pollen. Seed fertility was also rather high; it was 63.2 percent in self-pollinated plants and 74.8 percent in those cross-pollinated with diploids. The rate of transmission by selfing was 33.1 percent.

### 2. *Slender*

Trisomic plants of this type are also readily distinguishable from the diploid. Slenderness in all of the plant parts, as compared with those of the diploid, is characteristic of this trisomic, from which the name is derived. The leaf index is the largest (25.3) and stomatal guard-cell length is the shortest among the trisomics described. The first foliage leaf is very narrow which allows the trisomic plants to be readily identified. The leaves are slender, thin and mostly drooping. The culms and leaves of Slender are purple-colored, being especially at the later stages. The pollen fertility of this trisomic is 94.7 percent and the seed fertility about 62 percent. The transmission rate was 21.9 percent in selfing.

### 3. *Pale*

This type can be readily distinguished in the early seedling stage because the first foliage leaf of the trisomic plants is extremely twisted at its tip. The plant height is a little short. The plant color of this trisomic is paler than the other trisomics or the normal diploid throughout the whole growing period. The leaves are thin and drooping. The flag leaf is very small in size, especially in some hybrids. The leaf index is 17.7. As compared with diploids, the spikes are somewhat denser with shorter and thinner awns. The rachilla is so tiny that it is invisible macroscopically in some cases. The seeds are shrivelled, but seed fertility is relatively high (78 percent in selfed spikes). However, the pollen fertility is the lowest of all the trisomic types, that is, about 72 percent of good pollen. The rate of transmission is 26.6 percent in selfed progeny.

### 4. *Robust*

Robust is distinguishable from the other trisomics by its robust growth;

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1) Leaf index was calculated with the leaf just below the flag leaf.



wide, thick, dark-green leaves; wavy leaf margins; and thick stems. These characters become most pronounced at the later stages of plant growth. The leaf index is small (13.3). Purple plant color is hardly visible in any of the growth stages. Seeds are very shrivelled. Pollen fertility is 92.6 percent, while seed fertility is about 88 percent. The transmission is 28.7 percent in selfing.

#### 5. *Pseudo-normal*

This type most closely resembles the normal diploid. However, it can be readily distinguished from the normal diploid by the relatively smaller size of all its plant parts and also by reversely turn-overed and twisted leaves, even at the very early seedling stage. The leaf index is 16.6. Pollen and seed fertility are very high, being 93.6 percent in the former and 81 percent in the latter (selfed). The transmission rate is 29.5 percent in the selfed progeny.

#### 6. *Purple*

The trisomic plants of this type can be distinguished by their robust and coarse characteristics. They have thick, wide, coarse and dark-green leaves; thick stems; and semi-spreading or oblique plant habit. However, at the very early seedling stage this type has no distinctive traits by which it differs from the normal diploid. Purple color in the stems and leaves is striking during the growth period. The flag leaf is relatively large in size and coarse in texture with a few wrinkles in the middle portion. The leaf index is relatively small (14.0). The awns are not spread out but converge together at their tips. The broadened outer palea generally covers entirely the basal half of the kernel from both sides so that the rachilla in the ventral crease becomes invisible. Seeds are relatively wide and short. Pollen fertility was 96.5 percent, while seed fertility was about 80 percent in selfs and crosses with diploid. The rate of transmission is 22.2 percent in selfing.

#### 7. *Semi-erect*

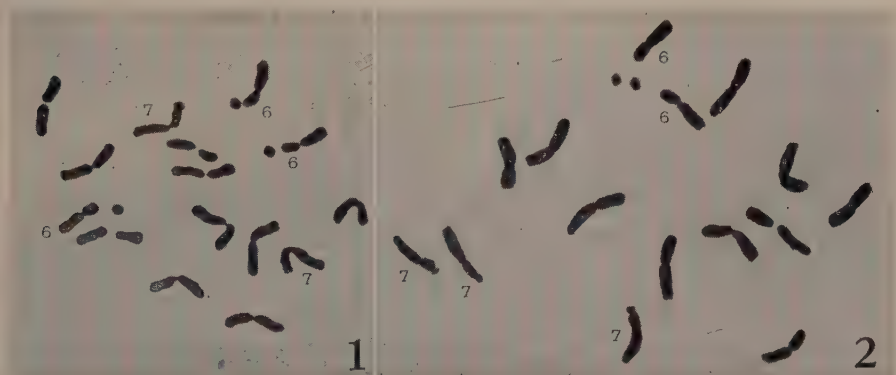
In the very early seedling stage, this trisomic type can be readily separated from diploids by its relatively small and narrow first foliage leaf. Its seedlings closely resemble those of the Bush trisomic, but the leaves are not so dark and tillers are fewer. This trisomic type is characterized by relatively short stems; short, wide, coarse and straight leaves; short and lax spikes; and the short and coarse awns; very long empty glumes. The leaf index is the smallest of all seven trisomic types, namely, 12.8. Seeds are the largest among all the trisomic types. The plant habit is semi-erect in the tillering stage as well as the younger stage. Pollen fertility was 96.6 percent, while seed fertility was 82.2 percent in selfs and 81.2 percent in ears crossed with diploid. The transmission rate is 31.3 percent in the selfed progeny.

As stated above, 5 out of the 7 trisomic types of barley here obtained are readily distinguishable from each other in their early seedling stages as well as from the diploid, even those with varied genetic back grounds. In the remaining two types, Robust and Purple, the coarse, robust and other distinctive fea-

tures mentioned above become clear at later growing stages. Thus all the seven trisomic types are characterized by distinctive and diagnostic features that readily distinguish them from the normal diploid.

The seven primary simple trisomic types just mentioned have been found repeatedly in the progenies of autotriploids and also in some double and triple trisomic plants derived from the autotriploid progenies (cf. [28, 29]).

Karyotype analyses have shown that Purple has chromosome 6 with the larger satellite (nucleolus chromosome or Burnham's *g*) in the triplicate condition (Fig. 1), whereas Semi-erect has chromosome 7 with the smaller satellite (Burnham's *d* chromosome) in the triplicate form (Fig. 2). Although in the other 5 trisomic types the morphological type of the extra chromosome has not yet been determined definitely, it has been shown that none of the 5 types had an extra SAT-chromosome.



Figs. 1-2. Somatic chromosomes of two trisomic types in *Hordeum spontaneum* var. *transcaspicum*. 1, Karyotype of Purple showing chromosome 6 with larger satellite in triplicate condition. 2, Karyotype of Semi-erect indicating chromosome 7 with smaller satellite in triplicate condition.  $\times 800$ .

Meiotic chromosome behavior at diakinesis and MI, especially the chromosome configurations and the types of trivalents, have been found to be different in each of all the seven trisomic types (Tsuchiya, unpublished).

All the cytogenetic findings here described, as well as many unpublished data, seem to support the independence of the seven primary simple trisomic types of barley as described above. The seven trisomic types used in the genetic experiments were shown by the study of somatic and meiotic chromosomes to have no other visible chromosomal aberrations.

## RESULTS OF GENETIC STUDIES

In 1955-1956, tests were made on the inheritance of 9 marker genes in the seven genetic linkage groups using the seven primary trisomic types. The original materials, *Hordeum spontaneum* C. Koch var. *transcaspicum* Vav., from which the trisomic types used in this study were obtained, is homozygous for the

TABLE I  
Marker genes, character pairs and their respective linkage groups involved in trisomics  
(*H. spontaneum* var. *transcaespitum*) and linkage testers

Linkage group	Character pairs	Analyzed genes in				
		Trisomic	Linkage testers			
		H.S.T. <sup>1)</sup>	Brachytic	A <sub>ca</sub> uzuz	Colless V	Minn 90-5
I	Non-six-rowed vs. six-rowed	V	v	v	v	— <sup>2)</sup>
II	Black vs. white lemma	B	b	b	b	—
III	Covered vs. naked caryopsis	N	n	n	—	—
IV	Hooded vs. awned	h	—	—	K	—
V	Long vs. short haired rachilla	S	—	s	s	—
V	Rough vs. smooth awn	R	—	—	—	r
VI	Normal vs. "uzu"	Uz	—	uz	—	—
VII	Normal vs. brachytic	Br	br	—	—	—
VII	Normal green vs. chlorina seedling	F <sub>o</sub>	—	—	f <sub>o</sub>	—

1) *Hordeum spontaneum* C. Koch var. *transcaespitum* Vav.

2) These characters are the same as those of trisomic parent or not tested.



8 dominant and one recessive normal (+) characters allelic to the 9 markers in the linkage testers. The genes for the investigated characters involved in four testers and the trisomics are shown in Table 1.

In all cross combinations, the trisomics were used as female parents and crossed with the 4 linkage testers shown in Table 1. Tests for segregation of the character pairs were made exclusively in the  $F_2$  generation of these crosses. As Rick and Barton [17] have pointed out, there are some good reasons for this procedure: selfing the  $F_1$  is easy with rare occurrences of contamination as compared with back-crossing; and the apparent accurate results are obtainable. Also somewhat smaller population is needed to discriminate between disomic and trisomic ratios, namely, 122 in  $F_2$  and 137 in the backcross, where no trisomic plants appear in the progenies.

The  $F_2$  seeds from the  $F_1$  trisomic hybrids were sown in a wooden box filled with sterilized soil. To facilitate later investigation, a preliminary test for the segregation of such characters as brachytic (*br*), uzu (*uz*), and chlorina seedling (*f<sub>c</sub>*) was made in the early seedling stage; then the normal and mutant plants were transplanted separately in the field. The same treatment was made, when possible, for the diploid and the trisomic plants. Thus, in some crosses such as Bush  $\times$  Colsess V, and Pale  $\times$  Colsess V, normal diploid, normal trisomic, diploid mutant, and trisomic mutant were separately grown in the field.

As already mentioned, the trisomic plants have distinctive and diagnostic features in the growing period under field conditions even with various genetic backgrounds. Consequently, the chromosome studies were made only of the doubtful individuals. In some cases, for example, Robust  $\times$  A.a.uzuz, in which the trisomic characters are not always clearly expressed in the early stage, almost all of the plants were examined cytologically.

The observed numbers as well as the  $\chi^2$  values found in each comparison with the numbers calculated for disomic ratios are shown in Table 2. Although the trisomic and the diploid plants were classified in each cross, for the sake of brevity, they are combined in this table.

As shown in Tables 2 and 5, all of the character pairs tested segregated in a simple Mendelian ratio of 3:1 in the diploids (Control).

Among the 56 cross combinations tested for  $F_2$  segregation of various character pairs, seven proved to deviate significantly from disomic ratios as shown in Table 2. In 44 cases the fit to the disomic expectation was very good. As to the 5 other cases, no  $\chi^2$  test was made because of small number. However, in two of them, namely, *Nn* in Pseudo-normal and *Ss* in Robust, it is improbable that the segregations fit a trisomic ratio, since an appreciable number of the trisomics were recessive (cf. [17]). Further studies are needed for those with small numbers.

As shown in Table 3, the seven populations that deviate significantly from disomic expectations segregated no trisomic recessive plants with the one exception of Semi-erect segregating for *Ss*, in which two homozygous recessive trisomics were observed among 47 trisomics. The actual numbers in these crosses were compared with those expected from trisomic segregation. The respective

TABLE 2  
Summary of segregation of 9 character pairs in the  $F_2$  generation of the crosses with seven trisomics

Types of trisomics	Items	Linkage groups and respective gene pairs																	
		I		II		III		IV		V		V		VI		VII		VII	
		+	v	+	b	+	n	K	+	+	s	+	r	+	uz	+	br	+	f <sub>c</sub>
Bush	Obs. number	279	104	288	82	186	13	120	52	137	35	123	36	99	21	98	3	172	11
	$\chi^2$ for 3:1	0.947		1.589		34.520		2.511		1.984		0.471		3.600		19.884		35.193	
Slender	<i>p</i>	0.50-0.30		0.20-0.10		very small		0.20-0.10		0.20-0.10		0.50-0.30		0.10-0.05		very small		very small	
	Obs. number	296	29	244	70	114	37	132	36	127	41	85	23	93	30	25	9	130	43
Pale	$\chi^2$ for 3:1	44.801		1.227		0.019		1.143		0.031		0.790		0.024		0.039		0.001	
	<i>p</i>	very small		0.30-0.20		0.90-0.80		0.30-0.20		0.90-0.80		0.50-0.30		0.90-0.80		0.90-0.80		0.98-0.95	
Robust	Obs. number	222	56	211	63	70	16	144	47	152	37	—	—	85	2	—	—	145	51
	$\chi^2$ for 3:1	3.496		0.588		1.876		0.015		2.965		—	—	22.771		—	—	0.108	
Pseudo-normal	<i>p</i>	0.10-0.05		0.50-0.30		0.20-0.10		0.95-0.90		0.10-0.05		—	—	very small		—	—	0.80-0.70	
	Obs. number	96	39	102	27	80	30	13	6	15	4	—	—	31	9	53	25	19	1
Purple	$\chi^2$ for 3:1	1.091		1.139		0.303		—		—		—	—	0.133		2.068		—	
	<i>p</i>	0.30-0.20		0.30-0.20		0.70-0.50		—		—		—	—	0.80-0.70		0.20-0.10		—	
Semi-erect	Obs. number	90	30	111	7	8	5	77	30	75	32	—	—	—		11	2	78	31
	$\chi^2$ for 3:1	0		22.881		—		0.502		1.374		—	—	—		—	—	0.688	
Diploid	<i>p</i>	—		very small		—		0.50-0.30		0.30-0.20		—	—	—		—	—	0.50-0.30	
	Obs. number	276	82	261	93	149	60	103	42	99	45	—	—	60	16	104	34	120	32
Pseudo-normal	$\chi^2$ for 3:1	0.838		0.305		1.532		1.216		3.000		—	—	0.631		0.009		1.263	
	<i>p</i>	0.50-0.30		0.70-0.50		0.30-0.20		0.30-0.20		0.10-0.05		—	—	0.50-0.30		0.95-0.90		0.30-0.20	
Semi-erect	Obs. number	274	77	255	81	141	42	109	47	145	11	—	—	94	25	64	17	122	35
	$\chi^2$ for 3:1	1.756		0.142		0.409		2.188		26.803		—	—	1.011		0.695		0.613	
Diploid	<i>p</i>	0.20-0.10		0.80-0.70		0.70-0.50		0.20-0.10		very small		—	—	0.50-0.30		0.50-0.30		0.50-0.30	
	Obs. number	180	56	153	70	95	25	81	21	80	22	76	18	44	15	68	26	82	20
Pseudo-normal	$\chi^2$ for 3:1	0.203		4.850		1.110		1.060		0.640		0.510	0.50-0.30	0.95-0.90		0.354		1.581	
	<i>p</i>	0.70-0.50		0.05-0.02		0.30-0.20		0.50-0.30		0.50-0.30		0.50-0.30	0.50-0.30	0.95-0.90		0.70-0.50		0.30-0.20	

TABLE 3  
 $F_2$  population that deviate significantly from disomic expectation

Types of trisomics	Linkage groups	Gene pairs	Diploid portion		Trisomic portion					
			Obsvd. no.	$\chi^2$ for 8:1	$p$	Obsvd. no.	$\chi^2$ for 44:1	$p$		
Bush	III	$Nn$	137	13	0.907	0.50-0.30	49	0	0.327	0.70-0.50
Bush	VII	$Brbr$	70	3	2.800	0.10-0.05	28	0	0.024	0.90-0.80
Bush	VII	$F_c f_c$	122	11	1.086	0.30-0.20	50	0	0.343	0.70-0.50
Slender	I	$Vv$	235	29	0.004	0.95-0.90	61	0	0.555	0.50-0.30
Pale	VI	$Uzuz$	58	2	2.933	0.10-0.05	27	0	0.017	0.90-0.80
Pseudo-normal	II	$Bb$	72	7	0.405	0.70-0.50	39	0	0.161	0.70-0.50
Semi-erect	V	$Ss$	100	9	0.899	0.50-0.30	45	2	0.287	0.70-0.50

TABLE 4  
Segregation of hooded vs. awned character ( $Kk$ ) in the diploid portion of  $F_2$  generation of trisomics  $\times$  Colless  $V$

Types of trisomics	Observed number		$\chi^2$ for 5:4	$p$	$\chi^2$ for 3:1	$p$
	$K$	+				
Bush	84	43	5.580*	0.02-0.01	5.314*	0.05-0.02
Slender	101	34	20.280**	very small	0.002	0.98-0.95
Pale	106	38	12.534**	very small	1.481	0.30-0.20
Robust	—	—	—	—	—	—
Pseudo-normal	46	23	3.450	0.10-0.05	2.556	0.20-0.10
Purple	65	24	11.010**	very small	1.835	0.20-0.10
Semi-erect	72	37	4.860*	0.05-0.02	4.651*	0.05-0.02

\* Significant at 5% level. \*\* Significant at 1% level.



TABLE 5  
Summary of results tested against disomic and/or trisomic ratio of 9 marker genes involved  
in respective linkage group

Types of trisomics	Marker genes in respective linkage group tested against								
	trisomic ratio			disomic ratio					
	I	II	III	IV	V	VI	VII		
Bush	<i>v</i>	<i>B</i>	*	—	<i>s</i>	<i>r</i>	<i>uz</i>	*	*
Bush	$\left. \begin{array}{l} n \text{ (III)} \\ br \text{ (VII)} \\ f_c \text{ (VII)} \end{array} \right\}$								
Bush									
Slender									
Pale	<i>v</i>	<i>B</i>	<i>n</i>	<i>K</i>	<i>s</i>	<i>r</i>	<i>uz</i>	—	<i>f_c</i>
Robust	<i>v</i>	<i>B</i>	<i>n</i>	<i>K</i>	<i>s</i>	—	*	—	<i>f_c</i>
Pseudo-normal	<i>v</i>	<i>B</i>	<i>n</i>	—	<i>s</i>	<i>r</i>	<i>uz</i>	<i>br</i>	—
Purple	<i>v</i>	*	—	<i>K?</i>	<i>s</i>	—	—	—	<i>f_c</i>
Semi-erect	<i>v</i>	<i>B</i>	<i>n</i>	<i>K</i>	<i>s</i>	—	<i>uz</i>	<i>br</i>	<i>f_c</i>
Diploid (Control)	<i>v</i>	<i>B</i>	<i>n</i>	—	*	—	<i>uz</i>	<i>br</i>	<i>f_c</i>
	<i>v</i>	<i>B</i>	<i>n</i>	<i>K</i>	<i>s</i>	<i>r</i>	<i>uz</i>	<i>br</i>	<i>f_c</i>

\* Trisomic ratio. — test not yet completed.

TABLE 6  
Relationships between trisomics and genetic linkage groups of barley

Types of trisomics	Trisomic ratios are observed in represented			Extra chromosome*
	genes	characters	linkage group	
Bush	<i>n</i>	Naked caryopsis	III	(1)
Bush	<i>br</i>	Brachytic	VII (III)	
Bush	<i>f<sub>c</sub></i>	Chlorina seedling	VII (III)	
Slender	<i>v</i>	Six-rowed	I	(2)
Pale	<i>uz</i>	Uzu (semi-dwarf)	VI	(3)
Robust	—	—	—	(4)
Pseudo-normal	<i>B</i>	Black kernel	II	(5)
Purple	—	—	—	6
Semi-erect	<i>s</i>	Short haired rachilla	V	7

\* Figures in parentheses are presumed from cytogenetic results of reciprocal translocations by Kramer et al. (1954) and Burnham (1957) as well as incomplete results of karyotype analysis by the present author. The others, 6 and 7, are determined by karyotype analysis by the present author.

$\chi^2$  values are shown in Table 3. In calculating the expected ratios random chromatid assortment was assumed as shown by Rhoades [16] and Rick and Barton [17]. As apparent in Table 3, good fit to the expected ratio were obtained in the diploid and in the trisomic portions.

In the cross with hooded Colless V, the trisomic parent carried the recessive genes and hence the  $F_1$  plants trisomic for the chromosome carrying this gene would be  $Kkk$ . Here the expected ratio is 29:16 or 35.6 percent recessives when the diploids and the trisomics are not separated. Further, when the diploids and trisomics are separated in  $F_2$  the expected ratios are 5:4 and 11:4 respectively. To discriminate the latter from a 3:1 ratio, over 600 trisomic  $F_2$  plants would be needed. The numbers here obtained are too small (see Table 2). Smaller numbers are needed for the diploid portion, about 93 to distinguish the 5:4 from a 3:1 ratio. The calculations here are made only for the diploid portions of the  $F_2$  data, as shown in Table 4.

In 3 of the 6 trisomic types tested, as seen from the  $\chi^2$  values in Table 4, the ratios for  $K:k$  deviated significantly from trisomic expectations and fitted very well the 3:1 ratio showing that the  $Kk$  gene pair was not carried by the extra chromosome of these 3 trisomic types, Slender, Pale and Purple. In Bush and Semi-erect, the deviations from both expected ratios were significant. In Pseudo-normal, the  $\chi^2$  values indicated good fits both for the trisomic (5:4) and disomic (3:1) expectations. Obviously, conclusions must be postponed until sufficient data are obtained with this character. An  $F_3$  test for  $Kk$  and  $F_2$  test for another gene pair,  $Bbl$ , both in linkage group IV, are now in progress with Robust (see Addendum, p. 28).

Based on the results mentioned above, the relationships between trisomic types and genetic linkage groups as well as the marker genes of barley are arranged as shown in Tables 5 and 6.

As clearly seen in Tables 5 and 6, the data obtained in the present experiments may allow the following conclusions:

1. The extra chromosomes in Slender, Pseudo-normal, and Pale carry genetic linkage groups I ( $Vv$ ), II ( $Bb$ ), and VI ( $Uzu$ ), respectively.
2. Bush carries on its extra chromosome two genetic linkage groups previously established, III ( $Nn$ ) and VII ( $Brbr$ ,  $Fef$ ), a result which is in accord with that suggested by Kramer et al. [13].
3. The extra chromosome of Semi-erect (chromosome 7 with the smaller satellite or Burnham's  $d$ ; Fig. 2) carries the genetic linkage group V ( $Ss$ ). This was already presumed without decisive evidence by Kramer et al. [13] and Burnham [3].
4. The extra chromosome of Purple (nucleolus chromosome or chromosome 6 with the larger satellite; Burnham's  $g$ ; Fig. 1) does not carry any one of the known genetic linkage groups, I-VII, which have been previously established by genetical methods (see Addendum, p. 28).
5. As mentioned above, Robust has not been studied sufficiently, while linkage group IV still has not been associated with any of the trisomic types. However, it was ascertained, as shown in Table 5, that Robust did not carry linkage groups I, II, III, VI and VII. Furthermore, in the cytogenetic studies of reciprocal translocations of barley, linkage group IV was most thoroughly in-



vestigated; the independence of group IV from the other 6 groups appears to be well established [3, 5, 6, 13] (see also Addendum, p. 28).

All the results described herein have led to the conclusion that the linkage groups in barley established by genetic methods is not 7 which was but 6. The other linkage group may be associated with the nucleolus chromosome with the larger satellite (Burnham's *g* chromosome).

## DISCUSSION AND CONCLUSION

Establishment of the relationships between individual chromosomes and genetic linkage groups of barley is certainly of scientific interest. Some attempts to determine these relationships have been made with considerable success [1, 2, 3, 5, 6, 8, 9, 10, 11, 13, 15]. Among them, the results obtained by Kramer et al. [13] are very important because they associated the two genetic linkage groups, III and VII, with only one chromosome. However, as pointed out by Robertson et al. [20], conclusive evidence from the linkage relations between the genes of these two linkage groups has not yet been obtained. This fact shows, in a sense, one of the limitations of the cytogenetic methods using linkages with reciprocal translocations as a test for independence (cf. [3]). Another is that the collection of the data and its analysis is more laborious in translocation methods (Hanson and Kramer [9, 10] and Joachim [11], and others).

The trisomic method is the most useful for these purpose, e. g. as was pointed out by McClintock [14] in maize and Rick and Barton [17] in tomatoes. Moreover, in the trisomic method, the collection and the statistical analysis of the data are also relatively simple. In spite of these advantages, the trisomic method has not been used in barley partly because of the difficulty in inducing trisomics. Ramage [15] has described genetic studies with trisomic barley that originated in plants heterozygous for a translocation. Many were tertiary trisomic types. The numbers obtained by him were low because fertility was greatly reduced.

The present author successfully obtained the expected seven types of primary simple trisomics in the progenies of autotriploid plants of a wild two-rowed species, *Hordeum spontaneum* var. *transcaspicum* [24, 25, 26, 27, 28, 29]. Fortunately, but unexpectedly, all have, in contrast to Ramage's stocks, relatively high seed fertility which facilitates genetic studies, especially in tests for  $F_2$  segregations in which the diploid parent carries the recessives.

The  $F_2$  tests for trisomic ratios have been carried out as shown in Tables 3 to 6. The experimental results were found to confirm the expectations: evidence obtained was very clear-cut and decisive. Direct and conclusive evidence for the two genetic linkage groups, III and VII, being on the same chromosome was obtained as seen in Tables 2 to 6. A gene pair, *Nn* (hulled vs. naked caryopsis), in linkage group III, and 2 gene pairs, *Brbr* (normal vs. brachytic) and *F<sub>c</sub>f<sub>c</sub>* (normal vs. chlorina seedling), of genetic linkage group VII, showed trisomic ratios in tests with the trisomic type, Bush. This finding decisively endorsed the results obtained by Kramer et al. [13]. Also the trisomic tests

furnish the conclusive evidence that linkage group V is associated with the Semi-erect trisomic type which is trisomic for chromosome 7 with the smaller satellite. Also the nucleolus chromosome with the larger satellite which appears 3 times in the trisomic type, Purple, was shown to carry no previously established genetic linkage group (see Addendum).

Thus, the trisomic method is shown to be excellent for testing the independence of linkage groups in barley, primarily due to the vigorous growth and the high seed fertility of the trisomic stocks here obtained. The relationships between each trisomic and of each of the genetic linkage groups are rather readily established as mentioned above. By using the trisomic method, also, it will be much facilitated to locate more genes especially new mutant genes on each chromosome of barley.

### SUMMARY

The major features by which the seven trisomic types may be distinguished from each other and from normal diploids are described. By means of trisomic inheritance, each trisomic type was tested against one or more genetic markers in each of the 7 linkage groups previously established by genetic methods. The results may be summarized as follows:

1. The extra chromosomes in the trisomic types, Slender, Pseudo-normal, Semi-erect, and Pale carry linkage groups of I (*v*), II (*B*), V (*s*), and VI (*uz*), respectively.
2. The extra chromosome of Bush carries the two genetic linkage groups, III (*n*) and VII (*br*, *f<sub>e</sub>*).
3. The extra chromosome of Purple (nucleolus chromosome or Burnham's *g*) carries no genetic linkage group previously known.
4. Tests with factors in linkage group IV are incomplete, but the independence of the group IV has been clearly shown by translocation methods [3, 13].
5. Based on present information, these results lead to the conclusion that the seven previously established linkage groups of barley are actually six.

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#### ADDENDUM

After the completion of this manuscript following informations have been obtained:

1. Genetic studies of *Kk* ( $F_3$ ) and *Blbl* ( $F_2$ ) gene pairs in Robust trisomic showed that these two gene pairs of linkage group IV were located on the extra chromosome of Robust (TSUCHIYA, T., HAYASHI, J., and R. TAKAHASHI. 1957. Further studies on the relationships between trisomics and genetic linkage groups in barley. Abst. 22nd Ann. Meet. Bot. Soc. Japan, held at Tokyo University, Tokyo, Japan, Oct. 12-15, 1957).

2. Kramer has located a gene for Xantha ( $X_n$ ) in the *g*-chromosome by use of translocations (Unpublished data; By personal communication from Dr. Burnham of the University of Minnesota, Minnesota, U. S. A.).

3. Ramage and Suneson (1958) has located a gene for earliness (*ec*) in the *g*-chromosome by testing the gene in 11 chromosomal interchanges (RAMAGE, R. T. AND SUNESON, C. A. *Agronomy Journal*, **50**: 114 (1958)).

From these findings the genetic linkage groups of barley became, again, to seven, that is, seven genetic linkage groups corresponding to the haploid number of chromosomes in barley was truly established.

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## LIFE-CYCLE OF A SPECIES OF *BATRACHOSPERMUM* FOUND IN NORTHERN KYUSHU, JAPAN<sup>1),2)</sup>

TADAO YOSHIDA<sup>3)</sup>

In a detailed investigation, Sirodot [12] confirmed that plants belonging to the genus *Batrachospermum* pass through a simple developmental stage called the *Chantransia*-stage, which is known not only in this genus but also in some related genera. He also stated that the carpospores formed as a result of sexual reproduction grew into the *Chantransia*-stage, and that the thallus of the *Chantransia*-stage reproduced itself asexually by monospores. Osterhout [9] observed the process of fertilization in *Batrachospermum boryanum*. Kylin [7] made cytological studies of *Batrachospermum moniliforme*, and assumed that reduction division took place in the carpogonium immediately after fertilization. He also described the germination of carpospores. In Japan, Saida [11] reported briefly on the life-cycle of *Batrachospermum* and confirmed the observations made by Sirodot. Geitler [3] also observed the germination of carpospores in *Batrachospermum* sp.

As stated above, the life-cycle of *Batrachospermum* has been clarified to some extent, but not all aspects of the supposed life-cycle have actually been established through culture experiments.

The author is happy to express his sincere thanks to Dr. S. Segawa of the Faculty of Agriculture, Kyushu University for his kind guidance throughout this investigation. Thanks are also due to Dr. R. F. Scagel of the University of British Columbia for his kindness in reading the original manuscript.

### MATERIALS AND METHODS

The material used in this investigation has not been identified as to species, but it is described in detail from both the morphological and the anatomical standpoints in the following section. The species of *Batrachospermum* studied was found in an irrigation stream flowing out of a well-spring at Hirao, in the suburbs of Fukuoka, northern Kyushu, and was growing on small stones and the roots of other plants. The seasonal changes in growth were observed mainly in this stream. Small pieces of glass plate were laid down in this stream and the development of the spores adhering to these glass plates was observed throughout the year. At the same time, plants of *Batrachospermum* growing in a small spring near the stream were also observed for comparison.

Culture experiments were carried out in the laboratories of Tobata High School in 1954 and Kyushu University in 1955, 1956 and 1957. In order to collect carpospores or monospores, a thallus of *Batrachospermum* bearing many ripe cystocarps, or stones covered with the thalli of the *Chantransia*-stage which were

1) Contribution from the Fisheries Laboratory, Faculty of Agriculture, Kyushu University.

2) Contribution No. 1, from "the Research Group on the Life-cycle of Red-algae" (Research Director—Dr. S. Segawa).

3) Fisheries Laboratory, Faculty of Agriculture, Kyushu University, Fukuoka, Japan.

previously ascertained to have ripe monosporangia, were brought into the laboratory. The fertile materials were placed in glass dishes or china vats filled with well-water and containing glass slides. After one or two days, spores became attached to the surface of the slides. Culture experiments of rather short term were carried out in standing water, which was replaced every 3-5 days, and those of long term in running water.

Cytological studies were carried out using the material collected from the lower part of the stream. The material was fixed chiefly in Flemming's weaker solution at the time it was collected in the field or after it was brought back to the laboratory. The aceto-carmin method as modified by Rao [10] was used for staining. The material was stained without sectioning. Rao's method is as follows:

1. Fix (Rao used acetic alcohol fluid).
  2. Treat about 10 minutes in iron alum solution.
  3. Rinse with distilled water (10 to 20 minutes).
  4. Stain in ordinary aceto-carmin solution.
- After staining, the material was squashed and observed.

## OBSERVATIONS

### 1. *Morphology of Batrachospermum used in this investigation*

The thallus reaches a length of 8-10 cm. and has a rather dense monopodial branching habit. It is light brown, sometimes rather bluish or greenish in colour, very mucilaginous, and adheres to paper when dried. This species is dioecious, but male and female plants are distinguishable only at maturity.

The thallus consists of a main axis bearing whorls of repeatedly branched primary laterals. Near the tip of the thallus, the adjacent whorls come in contact and are discoid in shape. In the proximal part of the axis the whorls, which are easily distinguished from each other, become thicker and sometimes nearly spherical in shape, giving a moniliform appearance. This condition is

especially true in the male plant<sup>4)</sup> (Fig. 1 A, B). The external appearance of the species varies to some extent according to the habitat.

The basal cells of the primary lateral give rise to rhizoidal filaments which cover the cells of main axis. Primary laterals ramify 6-10 times, and the ultimate branches of the lateral rarely terminate in hairs. When present the latter are short in length. Secondary laterals develop very rarely.

Male plants seem to be less

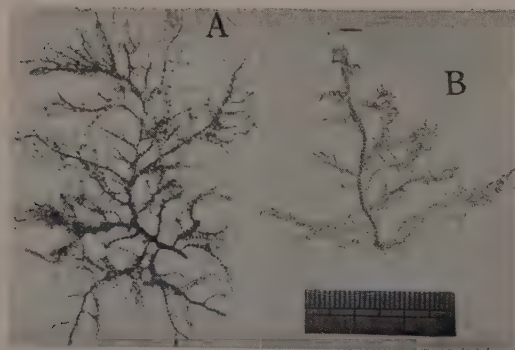


Fig. 1. *Batrachospermum* sp.  
A. Female plant. B. Male plant.

<sup>4)</sup> The specimens are deposited in the herbarium of Fisheries Laboratory, Faculty of Agriculture, Kyushu University.



abundant than female plants and are smaller in size. Spermatangia are formed at the tips of the laterals (Fig. 2 B). The laterals bearing spermatangia are richly branched, especially in the upper part. The spermatium is spherical,  $6-7\ \mu$  in diameter, and does not contain a chromatophore at maturity. A part of a chromatophore is present in the spermatium at the early stage, but it disappears in the course of maturing. The spermatium has no membrane when it is released, but develops a thin membrane after it becomes attached to a trichogyne.

The carpogonium is terminal on the carpogonial branch, which is hardly distinguishable from primary laterals in the early stage (Fig. 2 A, D). The carpogonial branch which consists of 10-16 cells, is almost equal in length to the

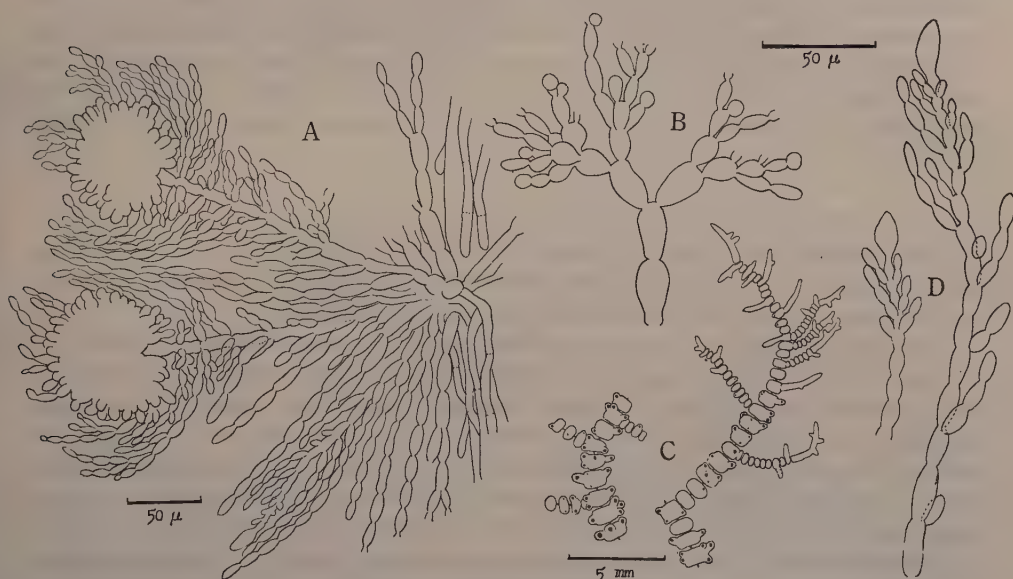


Fig. 2. A. A portion of whorl with carposporophytes. B. A part of lateral bearing spermatangia. C. Portions of the female plant showing the position of carposporophytes. D. Carpogonial branches.

laterals, so that the carpogonium is situated at the periphery of the whorl. Rarely a side branch of the carpogonial branch also bears a carpogonium. The trichogyne is ellipsoid in shape,  $25-30\ \mu$  long and  $7-9\ \mu$  wide (Fig. 2 D). After fertilization each cell of the carpogonial branch becomes enlarged and from these large cells project many short laterals (Fig. 2 A). The latter partly surround the carposporophyte, developing like whorls, and sometimes give rise to an elaborate structure with rhizoidal filaments as in the main axis. As a result, the carposporophytes may be situated outside or at the periphery of the whorl.

The characteristics of the species described above can be summarized as follows: 1) the secondary laterals develop very rarely, 2) hairs are very few in number, 3) the species is dioecious, 4) the trichogyne is ellipsoid in shape, 5) the carposporophyte is situated at the periphery or outside of the whorl, and 6) the

carpogonial branch gives rise to laterals in the form of whorls.

## 2. Seasonal changes of the thallus in natural habitats

The following description of seasonal changes in the thallus of this species of *Batrachospermum* is a result of observations made mainly in 1955.

The thalli of *Batrachospermum* were not observable throughout the summer. They attained their maximum growth from January to April, decreasing gradually thereafter, and vanishing completely in late June. Small buds of *Batrachospermum* could be seen in early November. They reached a height of 2–3 cm. by January, 1956. In a small spring near the stream, the thalli of *Batrachospermum* appeared earlier than in the stream. Duration of the thallus varied according to the habitats. This difference may be attributable to the water temperature. As the thalli of *Batrachospermum* disappear in summer, they are annual in this district, but it is said that the thalli of some species are found almost all the year round in Hokkaido. In Sweden, Kylin [5] reported that all species found there mature in summer months.

## 3. *Chantransia*-stage

The thalli of the *Chantransia*-stage form bushy patches or dense carpets on the substratum. Although they become several millimetres high when well developed, they develop poorly in the above-mentioned locality.

The thallus consists of prostrate and erect parts. The prostrate part, which covers the surface of the substratum, is composed of irregularly branched filaments of short creeping cells. The erect part, which is made up of uniseriate filaments, originates from the prostrate part, and when well-developed, ramifies several times. Cells of the erect filaments are 8–10  $\mu$  wide and 15–30  $\mu$  long, and each cell contains an irregular, parietal, yellow-green or yellow-brown chromatophore. Monospores are formed singly or in pairs at the tip of a side branch<sup>5)</sup>.

Differentiation of the thallus of *Batrachospermum* from the *Chantransia*-stage (Sirodot uses the term 'metamorphose') begins in November, and is seen till February of the next year in the above-mentioned stream. The thalli of *Batrachospermum* are formed as lateral buds on the filaments of the *Chantransia*-stage. The bud is distinguishable from the latter even in the very early stage. When the bud reaches a length of several cells, its cells divide longitudinally to form basal cells of the laterals, resulting eventually in the structure seen in the adult thallus (Fig. 3 A–C). When the young shoot of *Batrachospermum* reaches a size such that it is visible to the naked eye, rhizoidal filaments arise from the basal cell of the primary laterals and adhere to the substratum (Fig. 3 D).

For the purpose of observing the attachment of spores, several small pieces of glass plate were placed in the stream each month from March to July, 1955. The results obtained from a study of these plates are as follows.

In April, branched prostrate filaments were observed on the pieces of glass laid down in March, and one-celled erect filaments, which reached a length of

5) The author once observed in a culture inflated cells resembling the monosporangia formed on the creeping filaments, but could not confirm the nature of them because of the scarcity of the material.

several cells in May, were already present. No germings were observed in May on the glass plates submerged in April.

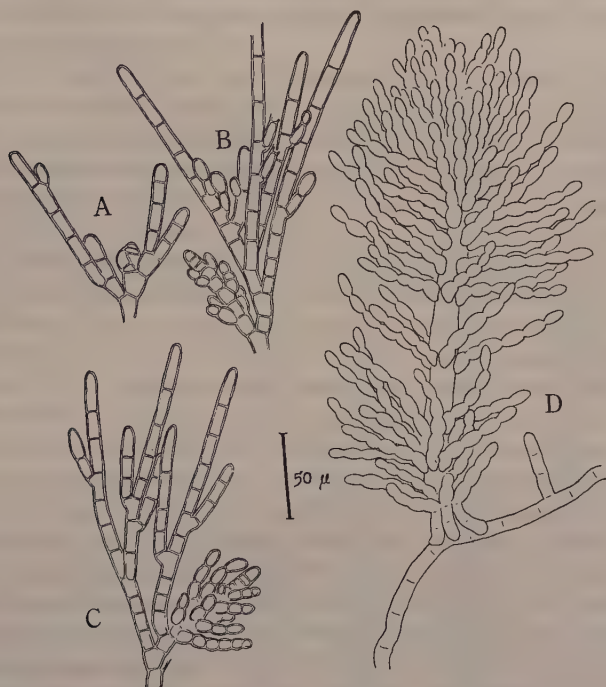


Fig. 3. A-D. Development of the thallus of *Batrachospermum* from the *Chantransia*-stage.

TABLE 1

Attachment of spores to glass plates laid down in the stream

		Date of observation							
Date of glass plates submerged		18/IV	13/V	4/VI	25/VI	14/VII	23/IX	6/XI	18/XII
	22/III	+	++	++	++	++	+	+	+
	18/IV		-	+	+	+	+	+	+
	13/V			+	+	+	+	+	+
	4/VI				+	+	-	+	+
	14/VII						+	+	+

- indicates the absence of germings

+ indicates the presence of germings

++ indicates the luxuriant growth of germings



According to observations made in June, well-developed germlings were found on the glass plates submerged in March. The erect filaments had reached a length of about 10 cells, and the densely ramified prostrate part had formed a somewhat discoid mat. No erect filaments were seen on the glass submerged in May.

Observations made in July revealed that on the glass plates laid down in March the cluster of germlings was more than 1 mm. in diameter and had formed yellow-green patches visible to the naked eye, although the erect filaments were without ramification. Relatively well-developed germlings were seen on the glass plates submerged in April, and some germlings were observed on the glass plates started in June.

In September, a black precipitate on the pieces of glass made the examination difficult, and it diminished the growth of the *Chantransia*-stage. The germlings were observed on the glass plates started in July.

Buds of *Batrachospermum* were found also on the pieces of glass in December, although the *Chantransia*-stage developed very poorly.

These observations include the germlings both from carpospores and monospores. The germlings from carpospores differ from those from monospores in the early stage, as described in the following section, but they are not distinguishable at a more advanced stage of development.

It seems probable that the germlings attached on the glass pieces laid down in July, and thereafter, might come from monospores because of the absence of *Batrachospermum* thalli in these months. The presence of monospores in these months is known from observation, although the *Chantransia*-stage bearing monospores was not discovered. It is probable that the *Chantransia*-stage propagates vegetatively, as suggested by Sirodot, but the author did not confirm this mode of propagation.

#### 4. Culture experiments with carpospores

At the time of liberation carpospores are spherical, 8–10  $\mu$  in diameter, and each carpospore has a single yellow-brown chromatophore (Fig. 4 A). Although the carpospores are naked when liberated, later they develop thin membranes. One or two days after liberation, carpospores begin to germinate. Sometimes they germinate close by the carposporophyte and entangled among the laterals of the mother plants. Kylin [7] and Geitler [3] observed a similar phenomenon in the germination of carpospores. It is uncertain whether or not spores that germinate on the mother thallus can later become adherent to the substratum, but the mode of germination seems to be identical with that on the glass slide.

On germination, a process arises from one side of the spore, and develops into a germ tube (Fig. 4 B, C). By the time the germ tube becomes twice as long as the diameter of original spore, most of the cytoplasm passes into this germ tube, and the latter is cut off by a septum at the slightly constricted basal part to form an initial cell of the germling (Fig. 4 D, E). The germ tube elongates, and by transverse division, initiates a uniseriate creeping filament (Fig. 4 F, G). When the germlings become 3–4 cells long, in many cases they give rise to

the first branch, which is formed from the cell nearest to the original spore, the initial cell (Fig. 4 H, I). Thereafter the branches originate from the other cells of the filament, forming an irregularly-branched prostrate part (Fig. 4 J). Each cell of the creeping filament contains parietal chromatophore of irregular shape.

In a culture experiment started with carpospores and using material collected from the spring, in July, 1956 the germlings, which grew in accordance with the mode of germination described above, gave rise to erect filaments. These erect filaments rarely ramified in summer months. Young shoots of *Batrachospermum* were discovered on the filaments of the *Chantransia*-stage in January, 1957. They gradually grew larger, and reached a height of about 3 millimetres in March. Although no thallus with a carposporophyte was found at this time, male individuals with mature spermatangia were seen. Thereafter, the growth of *Batrachospermum* diminished little by little. The shoots of *Batrachospermum* disappeared entirely in July, but the patches of the *Chantransia*-stage without monospores were still present. In this year, monospores were found on the filaments of the *Chantransia*-stage from January to April. These monospores were attached successfully on the other glass slides in March, and they germinated to form branched prostrate filaments following to the method described in the next section. These germlings were destroyed by a growth of diatoms after two months. It is evident from this experiment that the *Chantransia*-stage is not annual. The culture experiments described here are summarized in Fig. 5.

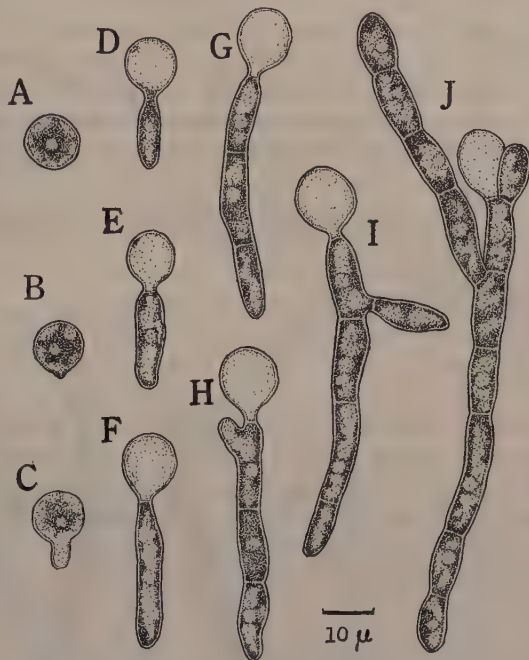


Fig. 4. Germination of carpospores.

A. Settled carpospore. B, C. 2 days old germlings. D, E, F. Formation of the initial cell, 6 days old. G, H. 10 days old germlings. I. Branching of prostrate filament, 14 days old. J. Germling of 30 days old.

##### 5. Culture experiments with monospores

Monospores are formed singly or in pairs on the tip of a side branch of the *Chantransia*-stage filament. They are about  $10\mu$  in diameter, and are naked when liberated, hardly distinguishable from carpospores (Fig. 6 A). One or two days after attaching to the glass slide, they begin to germinate, pushing out a germ tube from one side into which passes almost all the cytoplasm of original

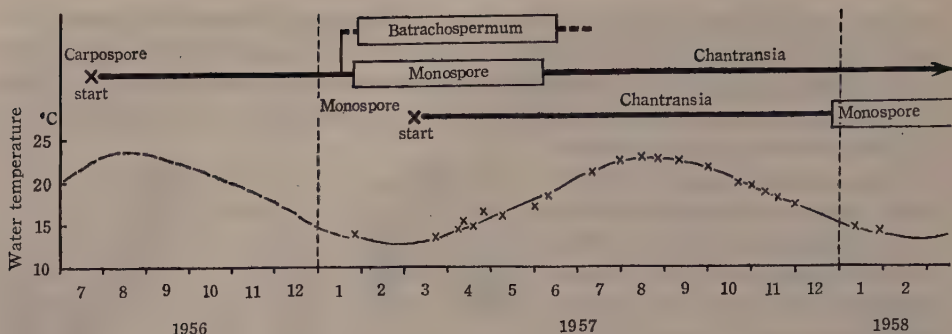


Fig. 5. Culture experiment with carpospores and monospores.

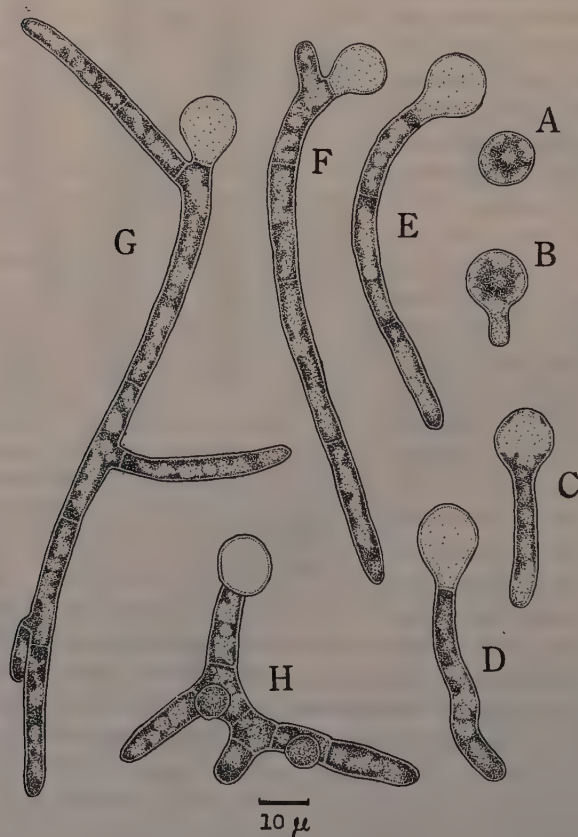


Fig. 6. Germination of monospores.

A. Settled monospore. B, C. 2 days old germlings. D. Formation of the initial cell, 5 days old. E. 8 days old germling. F. Ramification of prostrate filament, 14 days old. G. Germling of 30 days old. H. Germling with erect filaments.



spore (Fig. 6 B, C). Empty spore hulls become slightly larger in diameter. By the time the germ tube reaches a length 2-4 times the diameter of the original spore, the germling gives rise to a septum near the base and, following transverse division, becomes a uniseriate filament (Fig. 6 C-G). No constriction, however, is found at the base of the germ tube, as occurs in the germling from the carpospore. The chromatophore is the same as in the germling from the carpospore. The first erect filament originates from a 6-10 celled germling (Fig. 6 H). Later, the erect filament branches a few times. In the culture experiment carried out in 1957, the germlings continued throughout the summer in this state and formed monosporangia in December, although no buds of *Batrachospermum* were observed on them.

#### 6. Development of the carposporophyte and cytological observations

The cells of *Batrachospermum* are, as already shown by Kylin [7], always uninuclear. The nucleus, which has one large nucleolus, is about  $3-4\ \mu$  in diameter in the ordinary vegetative cell. The nuclear cavity is not stained with carmine or other dyes. In the prophase nucleus, small chromatin granules are visible.

The trichogyne has no chromatophore in it. The carpogonium is smaller than the trichogyne, and contains one nucleus. The trichogyne nucleus commonly reported in many other Floridean species has not been observed (Fig. 7 A). After the spermatium nucleus reaches the carpogonium, the membrane of the basal part of trichogyne becomes thicker, and eventually the connection between the carpogonium and the trichogyne is closed (Fig. 7 B, C). The trichogyne is apparent till the carposporophyte develops to some extent.

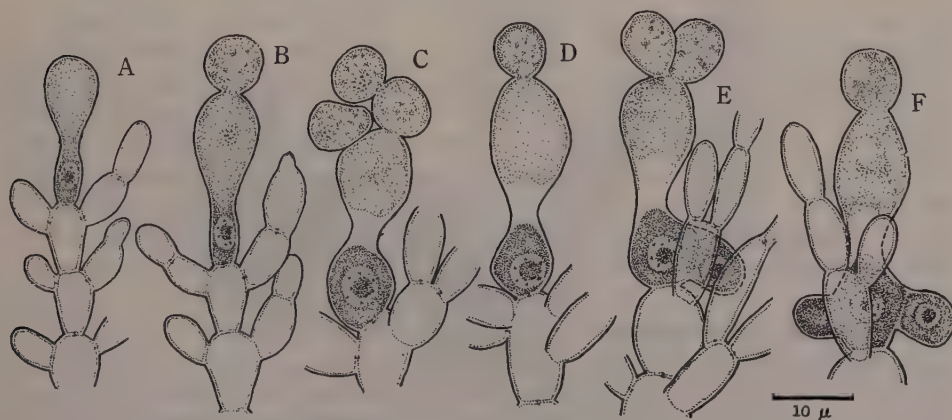


Fig. 7. Fertilization of carpogonium and early development of carposporophyte.

It might be expected that in the genus *Batrachospermum* the first division of zygote nucleus is meiotic, as in the other haplobiontic members of Nemalionales. However, the author was prevented from following the process of nuclear division by such difficulties as the small size of nucleus, and rare occurrence of

suitable material because of the proximity of the projecting bracteal branchlets arising from the supporting cells of carpogonium before and after fertilization. Since the state of diakinesis in the prophase nucleus was not ascertained, there is no direct evidence that meiosis takes place in the carpogonium.

The carpogonium gives rise directly to gonimoblast filaments. After fertilization the carpogonium grows larger, bulges out to one side, and cuts off a gonimoblast initial. Later a swelling is formed on the other side of the fertilized carpogonium (Fig. 7 D-F). It is believed that the four nuclei produced as a result of meiosis all take part in the development of gonimoblast filaments. The gonimoblast filaments gradually develop to form a compact mass with dense ramification, the carposporophyte. The terminal cell of the gonimoblast filament changes into a carposporangium which produces one carpospore at maturity.

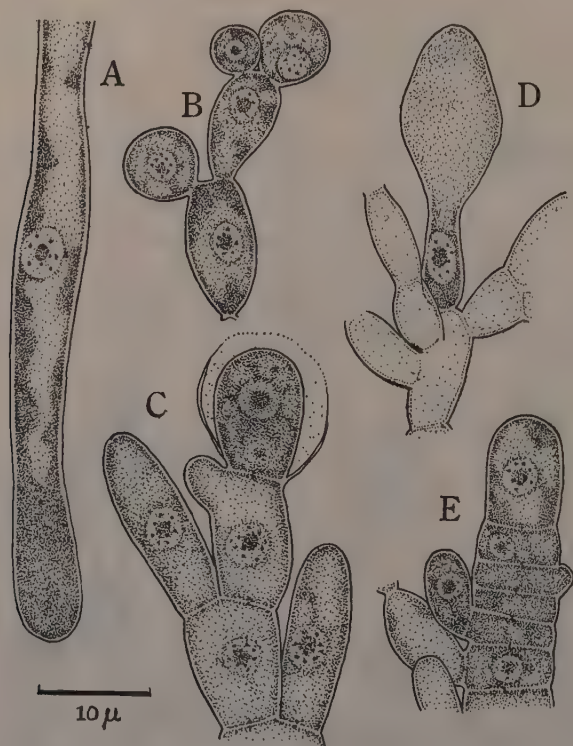


Fig. 8. Various portions of the thallus showing prophase nuclei.

The prophase nucleus in the cell of the gonimoblast filament contains chromatin granules which are similar in number to that in the nucleus of spermatium or vegetative cell (Fig. 8 A-E).

Simultaneous with the development of the gonimoblast filaments, each cell of carpogonial branch grows larger both in diameter and in length, and gives

rise to many short laterals, some of which surround the carposporophyte.

The spermatium mother cell is hardly distinguishable from other cells of the lateral, although sometimes it is somewhat larger in diameter. It is generally agreed that the nucleus in the young spermatangium is in the resting stage and this has been confirmed in the present study. The nucleolus disappears during maturation of the spermatangium, and at the same time some chromatin granules appear scattered through the nuclear cavity (Fig. 8 B). This state is interpreted as the prophase of a nuclear division, and it is suggested that the number of granules found in the nucleus of a mature spermatium coincides with the haploid chromosome number. It is difficult to count these granules precisely because of their small size and also because the mature spermatium contains many additional granules somewhat similar to them. The liberated spermatium is uninucleate. It cannot be determined from the author's preparations whether or not the spermatium nucleus undergoes a division after attaching to the trichogyne as Kylin [6] has described for *Nemalion multifidum*.

The most suitable place in which to observe the nucleus in the vegetative tissue is the terminal cell of the rhizoidal filaments covering the main axis. A similar number of chromatin granules is observed in these rhizoidal cells (Fig. 8 A).

## DISCUSSION

The germlings both from carpospores and monospores demonstrate the method of germination described by Inoh [4] as 'der mittelbare Fadentypus'<sup>6)</sup>, although the germling of the carpospore is distinguishable in the early stage from that of the monospore in having a slightly constricted part at the base of its germ tube. Both germlings give rise to branched prostrate filaments, with erect parts originating from them, and develop into the *Chantransia*-stage. Sirodot [12] distinguishes 'Prothalle' and '*Chantransia* (Form asexuée)' as early stages of *Batrachospermum*. Prothalle, according to him, consists of prostrate and erect parts, but the latter develop to a lesser extent, are microscopic in size, and very rarely form monospores. *Chantransia* grows to a length of about 1 cm., and its erect part is profusely branched and produces many monospores. The author is of the opinion that it is unnecessary to accept these obscure distinctions, but it is apparent that the shoots of *Batrachospermum* originate not so much from the well-developed *Chantransia*-stage as from that of a poorly developed one.

It is ascertained from culture experiments, as shown in the preceding section, that the *Chantransia*-stage developing from the carpospore produces monospores which germinate into a filamentous growth similar to the mother plant, and can produce the shoot of *Batrachospermum* in the same year. Although the author has not been able to obtain the shoot of *Batrachospermum* from the *Chantransia*-stage which originated from a monospore in culture, it may be assumed that the *Chantransia*-stage which came from monospore will give rise to the thallus of *Batrachospermum*. On the other hand, Saïda [11] says, in his brief report, that

6) The author quotes here his German term, which is considered to be correct.



he obtained the *Chantransia*-stage from carpospores of a species of *Batrachospermum* (he refers it to *B. coerulescens* Sirodot) and that monospores formed on the *Chantransia*-stage in culture grew into thalli similar to that of the mother plant.

It can be said with some certainty that the number of chromatin granules appearing in the prophase nucleus—believed to coincide with the chromosome number—is about 10 in all. As the author has not been able to observe all the stages in nuclear division, partly because of the smallness of nucleus, it is difficult to determine the exact chromosome number. Kylin [7] states that the number is about 10 in *Batrachospermum moniliforme*. Yamaha and Suematsu [14] report that there are Feulgen-positive granules in the nucleus of two species of *Batrachospermum*, i.e., *B. moniliforme* and *B. virgatum* (?), but they say nothing about the chromosome number. As the chromatin granules found in the nuclei of gonimoblast filaments, vegetative cells and spermatia are nearly similar in number, it seems fairly certain that meiosis takes place in the carpogonium immediately after fertilization, although there is no direct evidence. The species of *Batrachospermum* under study is, therefore, considered to be haplontic, as previously mentioned.

Before describing the life-cycle of the species of *Batrachospermum* under consideration from the above-mentioned facts, some attention must be given to the terms used in the interpretation of the phenomena for the general understanding of the life-cycle found in the algae.

During the early period of the study of the life-cycles of the algae, great emphasis was placed only on the nuclear phase, and it was considered that the morphological generation should be comprehended from the nuclear phase, as a result of the studies made by Svedelius [13] and others. More recently, Feldmann [2] and Drew [1], on the contrary, treat the nuclear phase and morphological phase separately, and Feldmann, using the term 'phase' for the nuclear phase and 'generation' for the morphological phase, considers the life-cycle as a combination of them. Drew, on the other hand, does not employ the term generation because it is difficult to define, and uses the term nuclear phase and somatic phase respectively.

The term 'stage' may be applied to a period in the life-cycle of an alga provided with distinct morphological characters as a developmental step. The *Chantransia*-stage of *Lemanea*, a genus related to the genus *Batrachospermum*, is believed to lack monospores, and be properly called as stage in its original meaning. On the other hand, in the multiaxial members of the Nemalionales, for example *Nemalionopsis tortuosa* Yoneda et Yagi (Okada and Migita [8]), germination of the spore is nearly the same as in *Batrachospermum*, and a prostrate part gives rise to an erect part, from which an adult thallus is formed without distinct morphological changes. Multiaxial Nemalionales are, therefore, considered to have no distinct developmental stage.

Drew [1] considers, with some hesitation, the *Chantransia*-stage of *Batrachospermum* as a somatic phase and gives it the name protogametophyte, because it shows constant and definite characteristic morphological features, produces re-

productive cells, the monospores, and its differentiation into the thallus of *Batrachospermum* is localized and sudden. But it would be rather difficult to refer to the *Chantransia*-stage of *Lemanea* as a stage and that of *Batrachospermum* as a generation or somatic phase, if we take into account that the difference between them is slight, except the existence of a reproductive organ in the latter. The author is, therefore, of the opinion that it should be referred to as a stage, and not a generation or somatic phase in the life-cycle. The number of generations or somatic phases would be considered as two, namely the thallus of *Batrachospermum* (including the *Chantransia*-stage) and the carposporophyte, and the life-cycle of *Batrachospermum* in question can be called diphasic, after Drew's terminology, or digénétique, according to Feldmann. The nuclear phase can be considered as monophasic (monophasique), because meiosis apparently takes place immediately after fertilization.

### SUMMARY

1. The germling arising from the carpospore differs from that from the monospore in having a constricted part at the base of its germ tube. Both germlings, however, show the same method of germination, 'der mittelbare Fadentypus' as defined by Inoh [4].
2. The *Chantransia*-stage of this species of *Batrachospermum* is observed at all times of the year, while the monosporangia are mainly produced from November to April of the following year. The thalli of *Batrachospermum* arise as lateral buds on the erect filaments of the *Chantransia*-stage.
3. Thalli of *Batrachospermum* can be seen from November to June of the following year, and are absent during the other months. In this district, therefore, the *Batrachospermum* described is an annual.
4. It is confirmed through culture experiments that the carpospore gives rise to the *Chantransia*-stage, which forms the thallus of *Batrachospermum*, and that monospores formed on the *Chantransia*-stage germinate and develop into the same stage. Monospores collected from natural habitats give rise to the same *Chantransia*-stage as in the case of carpospores.
5. Chromatin granules found within the nuclear membrane of prophase nuclei of the spermatium, gonimoblast and vegetative cells are almost similar in number. It is, therefore, assumed from this fact that reduction division takes place in the carpogonium immediately after fertilization.
6. The *Chantransia*-stage of this species of *Batrachospermum* is considered as a stage of development, and not as a distinct generation or somatic phase as interpreted by Drew.

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## CHROMOSOME STUDIES ON FOUR DIFFERENT SPECIES OF *CINNAMOMUM*

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AND

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### INTRODUCTION

*Cinnamomum* is one of the most important genera under the family Lauraceae. A number of its species yields materials which are used as spices, a commercially important oil, as well as camphor of high quality. *Cinnamomum tamala*, *C. zeylanicum* and *C. camphora*, which are cultivated for such purposes, grow profusely in India. They require moderately low temperatures and in India they particularly prefer subtropical and temperate climate. Of the species that are found in India, except in *C. camphora*, even the chromosome counts have not been reported in others. In *C. camphora*, the chromosome number only is known [5].

Uptil now only seven species of *Cinnamomum* have been investigated in total, all of which are characterized by having  $2n=24$  chromosomes in them [5, 6]. In this respect the genus, therefore, represents quite a homogeneous grouping. The basic number for the genus has been considered, to be  $x=12$ .

It is needless to mention that in the genera where all the species have the same chromosome number, karyotype studies prove to be very helpful (vide Stebbins [4]). In such cases, records show that the morphology of the chromosomes in the complement often differs from species to species and as such their karyotypes serve as an important tool in their classification.

In India, four species of *Cinnamomum* are available in Bengal, specially in the hilly areas. These are *C. camphora*, *C. tamala*, *C. zeylanicum* and *C. iners*. The first one is, however, cultivated on a large scale. In view of the fact that chromosome numbers in three of them and karyotypes of all are absolutely unknown yet, the necessity for their study was highly felt. As such an investigation may yield data indicating how far interspecific differences are associated with the karyotypic changes, the necessity for this line of work has been more and more realised. Taking all these factors into consideration, investigation on the different species of *Cinnamomum* had been undertaken and the following paper deals with the chromosome studies on four species of this genus and their possible significance indicated by the structure and behaviour of chromosomes.

### MATERIALS AND METHODS

#### 1. Materials

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The materials were obtained from the following species:

- a) *Cinnamomum camphora* T. Nees
- b) *C. zeylanicum* Nees
- c) *C. tamala* T. Nees
- d) *C. iners* Reinw.

The genus includes about one hundred and thirty species distributed in the tropical and subtropical areas of Eastern Asia, Australia and the Pacific. Several species occur in India, but in Bengal only a few cultivated ones are found.

The species were obtained from the local nursery and the College compound. Root-tips were collected from cuttings planted in pots.

## 2. Methods

Techniques, both permanent and temporary, were followed for somatic studies of chromosomes. Out of trials in various fixatives, a mixture of 1% chromic acid and 10% formalin (1:2) yielded good results. Best results were, however, obtained with pre-treatment chemicals followed by smearing. A saturated aqueous solution of Aesculin was found to be most advantageous [3]. Healthy root-tips were treated with the chemical for one hour at 4–6°C. The materials were then fixed in Acetic-Alcohol (1:1) for 45 minutes and then these were hydrolysed and stained in a mixture of 2% Aceto-orcein and N/HCl (9:1) by heating over a flame for a few seconds. Smearing was done in 1% Aceto-orcein solution and the preparations were sealed properly.

Sections of root-tips showed a large quantity of dark yellow cellular inclusions forming a thick layer in the meristematic zone and thus masking the divisional figures. These could not be removed without affecting staining. This trouble was, however, overcome in temporary smears.

For meiotic studies, flower buds were fixed in Navaschin's fluid A and B (1:1). A pre-treatment in Carnoy's fluid for a few seconds followed by thorough washing was necessary.

Paraffin sections were cut 14  $\mu$  thick and stained following Newton's crystal violet schedule. The peak periods of meiotic and mitotic divisions were between 10 A.M. and 1 P.M.

The figures were drawn at a table magnification of  $\times 2,900$  using a Zeiss compensating eye-piece of  $\times 20$  and a 1.3 apochromatic objective with a condenser of 1.3N.A. The chromosomes with secondary constrictions have been drawn in outline.

## OBSERVATIONS

All the four different species are characterized by constant chromosome number of  $2n=24$  in their normal somatic cells. Nuclei with varying number of chromosomes are found in most of the species, varying from 4 to 11% cases. The normal somatic number is considered as that which occurs in the highest frequency. The chromosomes show no marked size difference but a gradation in size from the longest to the shortest is always present within the complement. The number of secondary constrictions varies from four to eight. Size of the

chromosomes ranges between  $0.9\mu$  and  $3.4\mu$ , and on its basis three general groups can be seen, viz., comparatively long, medium and short. Primary constrictions are mostly median to submedian in position.

As much similarity in chromosome morphology amongst the species is noticeable, the general chromosome types are described first on a comparative basis. Their finer differences will be dealt with separately. The main types are:

Type A—Long chromosomes each with two constrictions, primary and secondary, one nearly median in position, and the other also nearly median to the comparatively shorter arm.

Type B—Long chromosomes having two constrictions, primary and secondary, one nearly median in position and the other placed in a nearly subterminal position at the distal end of the longer arm.

Type C—Long chromosomes with submedian primary constrictions.

Type D—Long chromosomes each possessing two constrictions located at the opposite ends. One is primary and the other secondary.

Type E—Long to medium-sized chromosomes with nearly median primary constrictions.

Type F—Medium-sized chromosomes with submedian to nearly submedian primary constrictions.

Type G—Medium-sized (nearly short) chromosomes each with a nearly median primary constriction and a satellite at the distal end of the slightly longer arm.

Type H—Medium-sized chromosomes each having two constrictions located at the opposite ends of the chromosome, at nearly submedian position, one primary and the other secondary.

Type H<sub>1</sub>—Similar to H type but comparatively much smaller in size.

Type I—Short chromosomes with nearly median to median primary constrictions.

Type J—Very short chromosomes with median primary constrictions.

Meiosis was worked out in only two species. In others flowers could not be collected. Meiotic irregularities are noted in both of them.

1. *Cinnamomum camphora* T. Nees ( $2n=24=4L^s+4L+2M^s+2S^s+12S$ )

A small tree. Leaves  $2\frac{1}{2}$ –4 inches long, glabrous, lanceolate, upper surface dark green shining, lower surface whitish. Panicles much shorter than leaves; flowers small.

The somatic chromosome number in the normal nuclei is found to be twenty-four (Fig. 1). Due to size difference within the complement the following groups can be recognized.

- a) Four pairs of long chromosomes,
- b) A pair of medium-sized chromosomes, and
- c) Seven pairs of short chromosomes.

Of them eight chromosomes are found to bear secondary constrictions. The size range varies between  $1\mu$  and  $3.4\mu$ . Detailed karyotype analysis is revealed from Table 1 and Fig. 2 (pp. 50).

Beside the normal karyotype, different complements with the same number



of twenty-four chromosomes show considerable structural changes in their chromosomes. These altered karyotypes are found even within the same root-tip (Figs. 3-4). Several other variation plates involving both numerical and structural alterations of chromosomes are also on record. These are somatic plates with twelve, seventeen, eighteen, twenty-two, twenty-five and thirty chromosomes (Figs. 5-10). Chromosomes with supernumerary constrictions are present in some of the variation plates (Figs. 7 and 10).

TABLE 1  
Karyotype analysis in *C. camphora* (cf. Idiogram on p. 50)

Type	Number	Special features
A	1 pair	Common A type
D	1 pair	Common D type
E	2 pairs	One pair slight short
H	1 pair	One constriction in each of the chromosomes is much pronounced
H <sub>1</sub>	1 pair	Common H <sub>1</sub> type
I	5 pairs	Chromosomes form a graded series
J	1 pair	Common J type

At diakinesis twelve clear bivalents have been observed (Fig. 11). First metaphase shows twelve clear bivalents in polar view (Fig. 12). Beside this normal meiotic behaviour, other irregularities are also noticed in some of the P.M.Cs. These include eleven bodies at first metaphase (Fig. 13), eleven, and thirteen bivalents at diakinesis (Figs. 14-15). Clear multivalents are also observed in some cases. The configurations are one trivalent, ten bivalents and one univalent; twelve bivalents and three univalents (Figs. 16-17). Meiotic irregularities are found to occur in 12% cases, of which in 9% cases clear multivalents are recorded.

## 2. *C. zeylanicum* Nees ( $2n=24=2L^s+2M^s+4M+16S$ )

A small evergreen tree. Leaves 4-7 inches long, glabrous, very coriaceous, ovate, 3-5 nerved. Panicles longer than the leaves, pubescent.

Twenty-four chromosomes are found to be present in the normal somatic

TABLE 2  
Analysis of karyotype in *C. zeylanicum* (cf. Idiogram on p. 50)

Type	Number	Species features
B	1 pair	Common B type
F	2 pairs	One pair slightly short
G	1 pair	Portion of the chromosome between the two constrictions is smaller than other parts
I	7 pairs	Common I type with gradation in size
J	1 pair	Common J type



Figs. 1-10. *Cinnamomum camphora* T. Nees. 1. Normal somatic metaphase ( $2n=24$ ). 3-4. Variation somatic metaphase with normal number ( $2n=24$ ) but involving distinct structural alterations of chromosomes. 5-10. Variation somatic metaphase with 12, 17, 18, 22, 25 and 30 chromosomes respectively.



Figs 11-17. *Cinnamomum camphora* T.Nees. Meiotic stages (for details, vide text). 16-17. Diakinesis showing trivalents, bivalents and univalents.  
 Figs. 18-29. *Cinnamomum zeylanicum* Nees. 18. Normal somatic metaphase ( $2n=24$ ). 20-21. Variation somatic metaphase with 24 (showing altered karyotype) and 26 chromosomes respectively. 22-29. Meiotic stages (for details, vide text). 23-24. Metaphase I with 10 and 11 bodies respectively.



cells (Fig. 18). Size difference is not marked and the chromosomes are generally medium-sized. Three groups, that can be seen, are:

- a) A pair of long chromosomes,
- b) Three pairs of medium-sized chromosomes, and
- c) Eight pairs of short to very short chromosomes.

Secondary constrictions are found in four chromosomes. The size ranges from  $1\mu$  to  $2.8\mu$ . Morphologically distinguishable types are shown in Table 2 (Fig. 19).

Altered karyotypes in the same twenty-four chromosome plates are observed in some nuclei (Fig. 20). Variation plates with twenty-six chromosomes are also seen (Fig. 21).

Meiosis is mostly normal. First metaphase reveals clear twelve bivalents in polar view (Fig. 22). Meiotic abnormalities observed are ten and eleven bodies at first metaphase (Figs. 23-24), early separation (Figs. 25-26) and lagging (Fig. 27). Second division is normal showing twelve chromosomes in each of the two poles (Fig. 28).

Another peculiar feature of the P.M.Cs. following first anaphasic separation of their chromosomes is the occurrence of numerous extranuclear bodies in their cytoplasm (Fig. 29). They are either freely scattered or associated in groups. Meiotic irregularities occur in 14% cases.

### 3. *C. tamala* T. Nees ( $2n=24=2L^s+2M^s+2M+18S$ )

A medium tree. Leaves 4-5 inches long, lanceolate, acuminate, 3-nerved, shining above.

$2n=24$  chromosomes are found to be the normal number (Fig. 30). Size difference is not pronounced and the following general groups are observed.

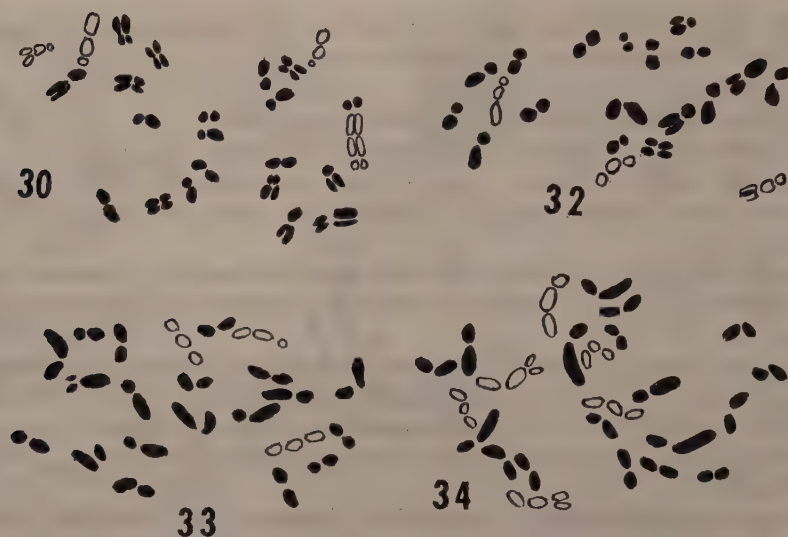
- a) A pair of long chromosomes,
- b) Two pairs of medium-sized chromosomes, and
- c) Nine pairs of short chromosomes.

Two pairs of chromosomes are characterized by secondary constrictions. The size difference varies from  $0.9\mu$  to  $2.7\mu$ . The following table shows the detailed karyotype analysis (Table 3, Fig. 31).

TABLE 3  
Analysis of karyotype in *C. tamala* (cf. Idiogram on p. 50)

Types	Number	Special features
B	1 pair	Common B type
F	1 pair	Common F type
G	1 pair	Common G type
I	8 pairs	Common I type
J	1 pair	Common J type

In addition to the normal karyotype described above, variation plates with twenty and twenty-two chromosomes are also recorded (Figs. 32-33).



Figs. 30-33. *Cinnamomum tamala* T. Nees. 30. Normal somatic metaphase ( $2n=24$ ). 32-33. Variation somatic metaphase with 20 and 22 chromosomes respectively. Fig. 34. *Cinnamomum iners* Reinw. Normal somatic metaphase ( $2n=24$ ).

2													
	A	D	E	E	H	H <sub>1</sub>	I	I	I	I	I	I	J
19													
	B	F	F	G	I	I	I	I	I	I	I	I	J
31													
	B	F	G	I	I	I	I	I	I	I	I	I	J
35													
	C	D	D	F	F	H	I	I	I	I	I	I	J

Figs. 2, 19, 31, and 35. Idiogram table of the different species of *Cinnamomum* so far investigated.

2. *C. camphora*. 19. *C. zeylanicum*. 31. *C. tamala*. 35. *C. iners*. Lettering of the chromosome types of the different species is prepared on a comparative basis.

4. *C. iners* Reinw. ( $2n=24=2L+4L^s+4M+2M^s+12S$ )

Leaves glabrous, shining above, 3-8 inches long, lanceolate-oblong, 3-nerved, nerves continued to the tip.

The normal somatic chromosome number for this species has been determined to be  $2n=24$  (Fig. 34). The chromosomes show size difference to some extent and on its basis three groups can be recognized.

a) Three pairs of long chromosomes, b) three pairs of medium-sized chromosomes, and c) six pairs of short chromosomes.

Secondary constrictions are present in three pairs of chromosomes. The size range varies between  $1.4 \mu$  and  $3.2 \mu$ . Table 4 shows the detailed karyotype analysis (Fig. 35).

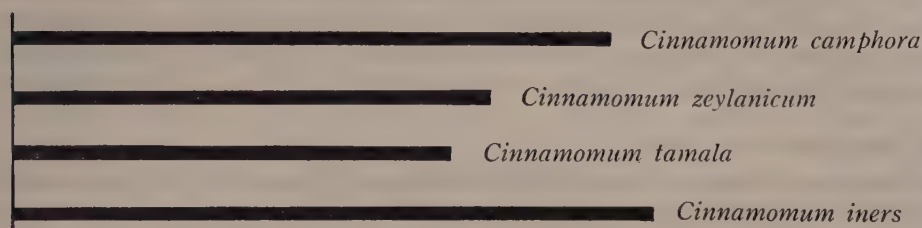


Fig. 36. Histogram showing the total length of chromatin matter in the haploid complement of the different species of *Cinnamomum* so far investigated.

TABLE 4  
Karyotype analysis in *C. iners* (cf. Idiogram, p. 50)

Type	Number	Special features
C	1 pair	Common C type
D	2 pairs	Portion of the chromosome between the two constrictions in one pair is slightly longer than in the other
F	2 pairs	Longer than common F type
H	1 pair	Common H type
I	5 pairs	Few pairs longer than common I type
J	1 pair	Longer than other J type

## DISCUSSION

1. *Chromosome numbers and the karyotypes*

Chromosome studies on four different species of the genus *Cinnamomum* mentioned in the text indicate marked resemblances in the morphology of the chromosomes amongst the species. All of them are characterized by mostly medium to short chromosomes and the size difference within the complement is



not very marked. Chromosomes are mostly provided with median to submedian primary constrictions. The number of secondary constrictions varies from four to eight in different species. The general homogeneity between them clearly indicates that the genus *Cinnamomum* indeed presents a natural assemblage of species.

Though in general, similarity in gross morphology of the chromosomes is noticeable, species differ with respect to minor karyotypic details. The number of secondary constrictions and types of chromosomes vary from species to species. Each and every species, therefore, can be distinctly identified on the basis of their karyotypes.

Such interspecific structural differences of chromosomes clearly indicate the role of structural alteration of chromosomes in the evolution of species. It is likely that through gradual evolution, homogeneity is attained by all the species so far as the structurally altered chromosomes are concerned. Clear bivalent formation during meiosis supports this assumption.

## 2. *Phylogeny of the species*

As regards the basic number for the genus, evidences so far accumulated indicate twelve as the basic number for all the species. In none of the species polyploidy has been noticed. In this genus, therefore, polyploidy seems to have played no role in evolution. No evidence of secondary association of bivalents too has been encountered, which if obtained, would have otherwise suggested their allopolyploid constitution.

In *C. camphora*, however, in 9% of pollen mother cells multivalent formation has been noted. One trivalent, ten bivalents and one univalent have been found in addition to the twelve bivalents in most of the P.M.Cs. The occurrence of such multivalent formation in this species in such a negligible percentage of mother cells (9%) can be accounted for on the basis of premeiotic irregularities as noted in the case of *Datura fastuosa* [1]. During premeiotic mitosis, some of the chromosomes of a particular type may fail to disjoin and go to a particular block of the daughter nucleus, whereas in the other sister nucleus, the same phenomenon may involve another type of chromosome. In addition to such nondisjunction, lagging may also account for the occurrence of univalents. Because of this process, in some of the daughter nuclei certain members of chromosomes may be represented more than twice, whereas this increase in number is compensated by certain deficiencies in some of the other types. The result is, therefore, the formation of nuclei where multivalent formations can be noted during meiosis, but otherwise maintaining a normal number of chromosomes. These nuclei though are similar to normal nuclei, so far as the chromosome number is concerned, their constitution is however different from the normal ones as regards the number of times each chromosome type is represented. Therefore, such multivalent formation in a negligible percentage of P.M.Cs. in species of *Cinnamomum* does not necessarily indicate that the species as a whole contains duplicated chromosomes. If this increase in chromosome types is not compensated by the deficiencies in certain other types, which is quite likely, as

the phenomenon occurs at random, the occurrence of P.M.Cs. with increased or decreased chromosome numbers is also expected. This behaviour has also been found to be the case in *C. camphora* and *C. zeylanicum*.

### 3. Karyotype alteration and its significance

Another important feature which has been found to occur in these species investigated here, is the presence of nuclei with varying number of chromosomes in the same somatic tissue (Table 5). They occur in about 4-11% of cells in the different species. Such varying numbers obviously arise through irregularities in nuclear division during mitosis. Such variations not only involve the number but also the structure of the chromosomes as well. This behaviour has been encountered in a large number of vegetatively reproducing plants and their significance in speciation has also been pointed out [2]. But in species of *Cinnamomum* where sexual reproduction is also effective, this behaviour cannot be regarded to be of much importance in effecting speciation. Further, in none of the species of *Cinnamomum*, biotypes with different chromosome numbers have been found which if obtained would have suggested the importance of such somatic irregularities in speciation aided through vegetative reproduction.

TABLE 5

Difference in chromosome morphology, variation and length of chromatin matter

Species	Normal somatic no. ( $2n$ )	Size difference in diploid complement ( $2n$ )	Variations in somatic number ( $2n$ )	Total length of chromatin matter in haploid complement
<i>Cinnamomum camphora</i>	24	$4L^s+4L+2M^s+2S^s+12S^{**}$	12, 17, 18, 22, 24*, 25 & 30	25.9 $\mu$
<i>C. zeylanicum</i>	24	$2L^s+2M^s+4M+16S$	24* & 26	20.5 $\mu$
<i>C. tamala</i>	24	$2L^s+2M^s+2M+18S$	20 & 22	18.9 $\mu$
<i>C. iners</i>	24	$2L+4L^s+4M+2M^s+12S$	—	27.8 $\mu$

\* Normal number but with structural alteration.

\*\* *L*-Long chromosome. *M*-Medium-sized chromosome. *S*-Short chromosome.

*L*<sup>s</sup>, *M*<sup>s</sup>, *S*<sup>s</sup>-Long, medium-sized or short chromosome with secondary constriction.

But in this connection it may be pointed out that structural variations of chromosomes in different complements, otherwise showing normal number, have also been noted in the somatic tissue. In view of the fact that vegetative propagation in the species of *Cinnamomum* too, is quite profuse in addition to sexual reproduction and also in view of the interspecific structural difference of chromosomes, it may be suggested that such somatic variations may play some role in the origin of the species of this genus—a process which is aided through their partly vegetative means of reproduction.

## SUMMARY

1. Four different species of the genus *Cinnamomum* have been cytologically studied. All the species are characterized by  $2n=24$  chromosomes. These are:

- |                                       |         |
|---------------------------------------|---------|
| a) <i>Cinnamomum camphora</i> T. Nees | $2n=24$ |
| b) <i>C. zeylanicum</i> Nees          | $2n=24$ |
| c) <i>C. tamala</i> T. Nees           | $2n=24$ |
| d) <i>C. iners</i> Reinw.             | $2n=24$ |

2. Karyotype analysis, in detail, has been performed in all the species. In spite of a gross similarity in their chromosome morphology, each and every species has got a karyotype of its own. The importance of this fact in identification has been pointed out.

3. Meiosis has been worked out in two species. In absence of any secondary association of bivalents and any polyploid species, twelve has been considered as the basic number for this genus. Multivalents and aneuploid meiotic numbers have been recorded in some P.M.Cs. Premeiotic irregularities have been considered responsible for such behaviour.

4. Nuclei with altered chromosome number have been observed in the somatic tissue of most of the species. As this genus maintains a constant chromosome number in its different species, the numerical variations cannot be regarded as of much importance in effecting speciation.

5. Nuclei with normal number of chromosomes but with structural alterations of chromosomes have been recorded in the somatic tissue of some of the species. As vegetative means of reproduction is also effective in species of this genus, these structural alterations seem to play some role in the origin of the different species.

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# NUCLEUS SUBSTITUTION AND RESTORATION BY MEANS OF SUCCESSIVE BACKCROSSES IN WHEAT AND ITS RELATED GENUS *AEGILOPS*<sup>1)</sup>

HIROSUKE FUKASAWA<sup>2)</sup>

## CONTENTS

I. Introduction.....	55
II. Materials .....	57
III. Nucleus exchange and its effect on pollen production .....	58
IV. Cytological and biochemical investigations on pollen degeneration in male-sterile plants .....	70
V. Characteristic appearances caused by foreign cytoplasm .....	75
VI. Cross-compatibility and embryo development in relation to foreign cytoplasm .....	80
VII. Summary .....	83
References .....	84

## I. INTRODUCTION

The effect of cytoplasm upon the phenotype is one of the most significant problem of genetical research. There are many publications dealing with cytoplasmic inheritance, although their number is far smaller than that of studies on nuclear genes or chromosomal factors. The most informative work in higher plants is that of Wettstein [159] on mosses, and Michaelis [99-111] and Lehmann [86, 87, 88] on various species of *Epilobium*. Recently, many suggestive findings have been reported concerning some micro-organisms; in regard to *Paramecium* by Sonneborn [141-145], concerning *Saccharomyces* by Ephrussi [27] and about *Neurospora* by Mitchell [112]. CO<sub>2</sub> sensitivity in *Drosophila* is also an interesting phenomenon caused by virus-like cytoplasmic element [91, 92]. Most of these informations have been summarized in several points of view in some articles [9, 17, 19, 28, 33, 74, 94, 138, 143, 157] and will not be detailed in the present paper. Certain facts on male sterility in higher plants, however, which is related closely to the present investigation, may be described briefly here.

A typical case, showing that cytoplasm possesses a factor which is responsible for the development of sex organ has been investigated by Correns [15, 16] in *Circium*. Following Correns's paper, many publications dealing with male sterility which could be interpreted by the plasmon theory or the interaction between cytoplasm and specific nuclear genes have been known; for example, in *Linum* by Bateson and Gairdner [6], Chittenden [11] and Gairdner [48], in *Zea mays* by

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Rhoades [127] and others [7, 23, 25, 57, 61, 134], in *Beta vulgaris* by Owen [120], in *Nicotiana* by East [24] and Clayton [12], in *Allium Cepa* by Jones and Clarke [59], in *Dactylis* by Myers [114], in *Saccharum* by Raghavan [125], in *Begonia* by Villerts [156], in *Secale* by Lein [89] and Sasaki [133], in *Solanum* by Koopmans [82], in *Petunia* by Frankel [30], in *Sorghum* by Stephence and Holland [149], in *Capsicum* by Peterson [122], in *Streptocarpus* by Oehlkers [116, 117], and in others which have been reviewed in detail by Edwardson [26].

Since the genome interrelations in the genus *Triticum* and its related genus *Aegilops* have been fully analysed by Kihara [66-74], this group is exceptionally suitable for the investigation of genom-plasmon relationships. Kihara and Yamada [79] reported, from the backcross experiments of  $F_1$  plants, *Ae. comosa* ♀ × *Ae. uniaristata* ♂, that only the gametes containing the perfect *comosa* genome were functional in *comosa* cytoplasm; while gametes with *uniaristata* genome aborted. Kihara and Kondo [76] described, in the reciprocal crosses between amphidiploid (*Ae. caudata* ♀ × *Ae. umbellulata* ♂) and *Ae. triuncialis*, that the *caudata* cytoplasm controlled the reduction in pollen fertility. Kihara [75] found pollen fertility to be influenced clearly by maternal cytoplasm, in successive backcrosses to the hybrid *Ae. longissima* ♀ × *Ae. Aucheri* ♂. Moreover, Kihara [75] obtained very interesting results in successive backcrosses to the intergeneric hybrid *Ae. caudata* × *T. vulgare*. When the complete genome of *vulgare* (VV) is introduced into the *caudata* cytoplasm ( $\alpha^c$ ), the plants become sterile. But, if a gene for black ear from *caudata* (or whole chromosome) is included in the *vulgare* nucleus the  $V^bV^b$  plants are fertile (70%). Thus, Kihara presented a systematic method of nucleus substitution and restoration through successive backcrosses. Kimura [80], according to Kihara's method, calculated the speed of the substitution for the plants with seven pairs of chromosomes.

My intention was to work with a hybrid or an amphidiploid whose genome complements consisted of strikingly non-homologous chromosomes. My present study, following Kihara's scheme, had been started in 1947, using two amphidiploids between two diploid species, *Ae. bicornis* and *Ae. squarrosa* [cf. 97], on the one hand and between two tetraploid species, *Ae. ovata* and *T. durum* on the other. The results obtained with the former amphidiploid contributed no very useful information for the present purpose. However, highly interesting results have been obtained from the experiments with the latter amphidiploid, *Ae. ovata* ♀ × *T. durum* ♂, which is generally called *Aegilotriticum* [cf. 155]. Some important portions of the results have already been reported under the title of "Studies on restoration and substitution of the nucleus in *Aegilotriticum*", and in other papers. The present paper deals with the final analyses of these findings and additional informations obtained from further experiments.

The author wishes to express his cordial thanks to Dr. H. Kihara, National Institute of Genetics of Japan, for his kind guidance and valuable suggestions during the present study, and to Dr. I. Nishiyama, Laboratory of Genetics, Kyoto University, for helpful advice throughout this work. Thanks are also due to Dr. S. Kusunoki, Dr. S. Fujii and Dr. H. Hirose who kindly allowed the author to complete this investigation at the Kobe University. The author also is indebted to Dr. H. G. duBuy, National Institute of Allergy and Infectious Diseases, U.S.A., who was kind enough to read over the manuscript and make some corrections in the style.

## II. MATERIALS

As mentioned above, the author used mainly such materials as an amphidiploid, consisting of different parental species with non-homologous genomes, since the  $F_1$  hybrid between their parents have produced frequently unreduced gametes.

TABLE 1  
List of materials used in the present study

Species name	2n	Genome formula	Abbreviation
<i>Triticum monococcum</i> var. <i>vulgare</i>	14	AA	<i>monococcum</i>
<i>T. aegilopoides</i> var. <i>boeoticum</i>	14	"	<i>aegilopoides</i>
<i>T. durum</i> var. <i>Reichenbachii</i>	28	AABB	<i>durum</i> (R)
" var. <i>hordeiforme</i>	28	"	<i>durum</i> (h)
" var. <i>coerulescens</i>	28	"	<i>durum</i> (c)
<i>T. dicoccoides</i> var. <i>Kotschyianum</i>	28	"	<i>dicoccoides</i> (K)
" var. <i>spontaneo-nigrum</i>	28	"	<i>dicoccoides</i> (sp)
" var. <i>straussianum</i>	28	"	<i>dicoccoides</i> (st)
<i>T. dicoccum</i> var. <i>liguliforme</i>	28	"	<i>dicoccum</i> (l)
" var. (Emmer)	28	"	<i>dicoccum</i> (E)
" var. (Russian)	28	"	<i>dicoccum</i> (R)
" var. <i>arras</i> (Khapli)	28	"	<i>dicoccum</i> (Kh)
<i>T. orientale</i>	28	"	<i>orientale</i>
<i>T. persicum</i> var. <i>stramineum</i>	28	"	<i>persicum</i> (s)
" var. <i>fuliginosum</i>	28	"	<i>persicum</i> (f)
" var. <i>rubiginosum</i>	28	"	<i>persicum</i> (r)
<i>T. turgidum</i> var. <i>nigro-barbatum</i>	28	"	<i>turgidum</i>
<i>T. pyramidale</i> var. <i>ricognitum</i>	28	"	<i>pyramidale</i>
<i>T. polonicum</i> var. <i>vestitum</i>	28	"	<i>polonicum</i>
<i>T. Spelta</i> var. <i>Duhamelianum</i>	42	AABBDD	<i>Spelta</i> (D)
<i>T. vulgare</i> (aestivum) var. <i>erythrospermum</i>	42	"	<i>vulgare</i> (e)
" var. <i>ferrugineum</i>	42	"	<i>vulgare</i> (f)
" var. <i>alborubrum</i>	42	"	<i>vulgare</i> (a)
" Norin No. 26	42	"	Norin 26
<i>T. compactum</i> var. <i>icterinum</i>	42	"	<i>compactum</i>
<i>Aegilops ovata</i> No. 1	28	C <sup>u</sup> C <sup>u</sup> M <sup>o</sup> M <sup>o</sup>	<i>ovata</i>
<i>Ae. bicornis</i> var. <i>typica</i>	14	S <sup>b</sup> S <sup>b</sup>	<i>bicornis</i>
<i>Ae. squarrosa</i> var. <i>typica</i>	14	DD	<i>squarrosa</i>
<i>Ae. ventricosa</i> var. <i>comosa</i>	28	DDM <sup>v</sup> M <sup>v</sup>	<i>ventricosa</i> (c)
" var. <i>fragilis</i>	28	"	<i>ventricosa</i> (f)
<i>Ae. variabilis</i> var. <i>intermedia</i>	28	C <sup>u</sup> C <sup>u</sup> S <sup>v</sup> S <sup>v</sup>	<i>variabilis</i>
<i>Ae. trinucialis</i> No. 1	28	C <sup>u</sup> C <sup>u</sup> CC	<i>trinucialis</i>
<i>Aegilotriticum</i> No. 2, by Tschermak and Bleier	56	C <sup>u</sup> C <sup>u</sup> M <sup>o</sup> M <sup>o</sup> AABB	<i>Aegilotriticum</i>
<i>Aegilotriticum</i> , by Fukasawa	56	"	<i>Aegilotriticum</i> (F)
Amphidiploid of <i>bicornis</i> × <i>squarrosa</i>	28	S <sup>b</sup> S <sup>b</sup> DD	Amphidiploid (SD)



As substitution and restoration were successful in the experiment with *Aegilotriticum* No. 2, which synthesized from the hybrid *Ae. ovata* ♀ × *T. durum* var. *Arraseita* ♂, further detailed investigations were carried out mainly with *Ae. ovata* and *T. durum* and its related species.

### III. NUCLEUS EXCHANGE AND ITS EFFECT ON POLLEN PRODUCTION

#### 1. Nucleus exchange between *T. durum* and *Ae. ovata*

Substitution and restoration of nucleus (strictly speaking, whole genome) were performed clearly in various conditions between *T. durum* and *Ae. ovata* [35, 37, 39]. Highly interesting results were obtained from the following several series of experiments, particularly regarding to male sterile condition.  $\alpha$  and  $\beta$ , which presented in next sentence, represent the cytoplasm of *ovata* and *durum* plants, respectively. (a) Placing of *durum* genome in *ovata* cytoplasm, using *Aegilotriticum* as the female parent; i.e.,  $(\alpha \text{ ovata} + \text{durum}) \text{♀} \times \text{durum}^n \text{♂}$ . (b) Placing of *durum* genome in *ovata* cytoplasm, using pure *ovata* plants as the female parent;  $(\alpha \text{ ovata} \times \text{durum}) \text{♀} \times \text{durum}^n \text{♂}$ . (c) Restoration of *durum* genome from the substitution line with *ovata* cytoplasm;  $\beta \text{ durum} \text{♀} \times [(\text{ovata} + \text{durum}) \times \text{durum}^n] \text{♂}$ . (d) Placing (restoration) of *ovata* genome into *Aegilotriticum*;  $(\alpha \text{ ovata} + \text{durum}) \text{♀} \times \text{ovata}^n \text{♂}$ . (e) Restoration of *ovata* genome into the *ovata* cytoplasm of MS *durum* plants;  $[(\alpha \text{ ovata} + \text{durum}) \times \text{durum}^n] \text{♀} \times \text{ovata}^n \text{♂}$ . (f) Placing (restoration) of *durum* genome into the hybrid, *durum* ♀ and *ovata* ♂;  $(\beta \text{ durum} \times \text{ovata}) \text{♀} \times \text{durum}^n \text{♂}$ . (g) Placing (restoration) of *durum* genome into the amphidiploid of *durum* ♀ and *ovata* ♂;  $(\beta \text{ durum} + \text{ovata}) \text{♀} \times \text{durum}^n \text{♂}$ . (h) Placing (substitution) of *ovata* genome in *durum* cytoplasm;  $(\beta \text{ durum} \times \text{ovata}) \text{♀} \times \text{ovata}^n \text{♂}$ . (i) Restoration of *durum* genome into the *ovata* plants with *durum* cytoplasm;  $[(\beta \text{ durum} \times \text{ovata}) \times \text{ovata}^n] \text{♀} \times \text{durum} \text{♂}$ .

In the experiments (a) and (b), all resulting 28-chromosome plants were thought to be plants with complete *durum* genome. Meiosis in the pollen mother cells proceeds normally, showing 14<sub>II</sub> at MI (MI represents metaphase of the first meiotic division). The microspore, however, fail to develop and disintegrate by the time the florets open. On the other hand, the female organs are normal. There is no evidence for abnormal preferential segregation of meiotic chromosomes at the megasporogenesis [cf. 95, 130]. No apomixies have been observed. Therefore, this must be considered as a case of cytoplasmic male-sterility. The male sterile condition has been maintained completely unchanged in further generations backcrossed with *durum* pollen. Thus, MS *durum* (MS represents "male-sterile") plants were established.

On the other hand, the experiments (c) (d) (e) (f) (g) (h) and (i) showed that all resulting plants became normally fertile in both male and female side. Morphologically they were also identical with the normal plants, except that  $\beta \text{ ovata}$  plants (h) showed some retardation in vegetative growth.

From the data obtained in these experiments, the processes and end results can be drawn schematically by the following diagram (Fig. 1). It should be understood, that the diagram represents ten possible experiments. Among these,

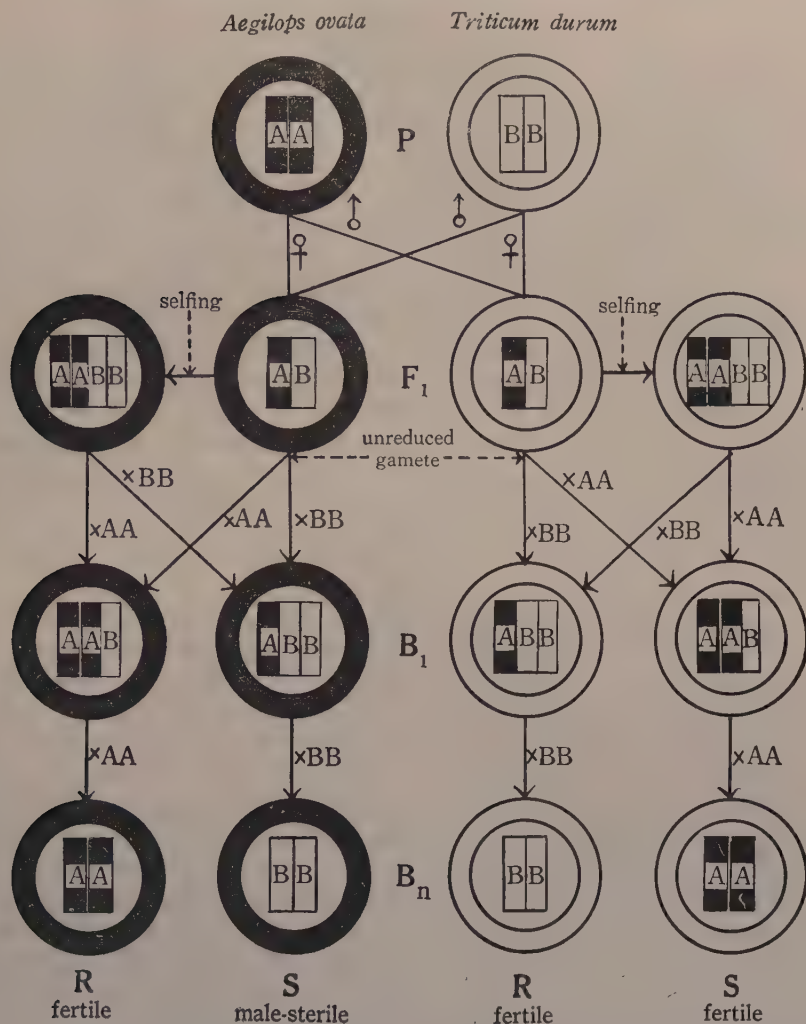


Fig. 1. Diagrammatic representation of the substitution and restoration of genome complements between *Aegilops ovata* and *Triticum durum*.  $\blacksquare$  and  $\square$  represent haploid set of *ovata* and *durum* genome complements. The black area denotes *Ae. ovata* cytoplasm.

$(\alpha \text{ ovata} \times \text{durum}) \varnothing \times \text{ovata}^n \sigma$  and  $(\beta \text{ durum} + \text{ovata}) \varnothing \times \text{ovata}^n \sigma$  remains to be determined. The results obtained in the experiments thus far, however, indicate that they will be in agreement with the present diagram. F<sub>1</sub> hybrids between *ovata* and Emmer wheats had 28 univalents in the majority of PMCs (Fig. 2). 1-3 bivalents were found in some cases (cf. 68). Unreduced gametes were produced by the formation of restituted nuclei. Consequently, the chance of cross-over between chromosomes of *ovata* and *durum* is very rare. Even if it would occur, the chromosome would be eliminated by backcrosses

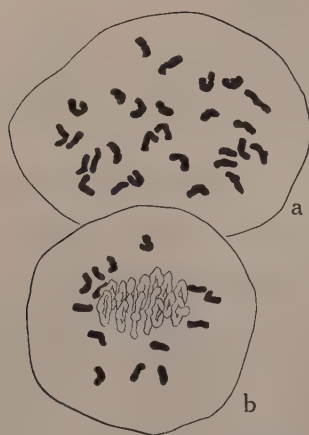


Fig. 2. Chromosome configurations in the first meiotic metaphase of the  $F_1$  hybrid, *ovata*  $\times$  *durum* and the back-cross plants. a, 28I. b,  $14_{II}+14_I$ . ca.  $\times 800$ .

with the pollen, which possesses the genome complements expected to be transferred. Further, by breeder's selection, using external morphological characteristics, the speed of genome transfer would also be increased. Actually, in the present experiments on the transfer of *durum* genome into *ovata* cytoplasm, all individuals possessing  $14_{II}$ -configurations showed male-sterility, without exception. Therefore, it may be concluded that all  $14_{II}$ -plants ( $14_{II}$ -plant means the plant containing 14 pairs of meiotic chromosomes) have not any unsubstituted chromosome segment, or that even if such unsubstituted portion would be present, it may not have been involved in pollen production.

## 2. Experiments with crosses between male-sterile *durum* and other related wheats

### a. Crosses between MS *durum* and other Emmer wheats

The Emmer wheats used as follows; *dicoccum* (E), *turgidum*, *dicoccum* (R), *orientale*, *dicoccum* (Kh), *persicum* (s), *polonicum*, *Pyramidale*, *dicoccoides* (sp), *dicoccoides* (st), *durum* (c), *persicum* (f). All the hybrids from these crosses showed  $14_{II}$  at MI and became male-sterile like the mother parent [37]. Further successive backcrosses with these Emmer wheats have established, that all lines were male-sterile. However, the lines crossed to *dicoccum* (E) plant matured so late in the further successive backcrosses that the crossing with normal *dicoccum* (E) became almost impossible. The same situation occurred in successive crosses, where late-maturing species as *dicoccum* (R) had been used as pollen parents.

It is, however, remarkable that the cross between MS *durum* and *dicoccoides* (K) produced only fertile offspring. The plants appeared somewhat weak and, strikingly, matured later than the other male-sterile lines. The chromosome configurations at MI were  $14_{II}$ ,  $13_{II}+2_I$ ,  $1_{IV}+12_{II}$  and  $1_{IV}+11_{II}+2_I$ . At first glance, it seemed that the deviating chromosome configurations are connected with pollen restoration. However, the hybrids with *dicoccoides* (sp) possessed such configurations as  $1_{IV}+12_{II}$  and  $1_{IV}+11_{II}+2_I$ , in spite of complete male-sterility. Moreover, in further generations from the fertile hybrid between MS *durum* and *dicoccoides* (K), male fertile plants with only  $14_{II}$ -configuration occurred. Therefore, it may be concluded that the irregular configurations are not connected immediately with pollen restoration.

### b. Crosses between MS *dicoccum* (Kh) and *dicoccoides* (K)

As mentioned above, hybrids from MS *durum*  $\times$  *dicoccoides* (K) matured so late and were so weak (some individuals died before heading), that investigations with sufficient numbers have not been carried out. MS *dicoccum* (Kh) was used in the next crossing experiment with *dicoccoides* (K). As expected their  $F_1$  hybrids showed high pollen fertilities (Table 2).



TABLE 2  
Chromosome configurations and pollen fertility in the  $F_1$  hybrids between  
MS plants and *dicoccoides* (K)

Combination	Pollen fertility %	Chromosome configuration
MS <i>durum</i> $\times$ <i>dicoccoides</i> (K)	79.0 $\pm$ 8.1	14 $\Pi$ , 13 $\Pi$ +2 $I$ 1 $IV$ +11 $\Pi$ +2 $I$ , 12 $\Pi$ +4 $I$
MS <i>dicoccum</i> (Kh) $\times$ <i>dicoccoides</i> (K)	97.2 $\pm$ 3.1	14 $\Pi$
[MS <i>durum</i> $\times$ <i>dicoccum</i> (E)] $\times$ <i>dicoccoides</i> (K)	80.1 $\pm$ 9.2	14 $\Pi$ , 1 $IV$ +12 $\Pi$ , 13 $\Pi$ +2 $I$ , 1 $IV$ +11 $\Pi$ +2 $I$
MS <i>durum</i> $\times$ <i>dicoccoides</i> (sp)	0	1 $IV$ +12 $\Pi$
MS <i>durum</i> $\times$ <i>dicoccoides</i> (st)	0	14 $\Pi$

Much offspring was easily obtained by selfing, or by backcrossing the offspring with each parents. In order to elucidate the behavior of pollen restoring factor, the  $F_2$ ,  $F_3$  and the backcross-plants were grouped into five classes according to the degree of their pollen fertilities (Table 3). The data suggest that

TABLE 3  
Frequency of the individuals grouped to five classes according to the degree of  
pollen fertility in the  $F_2$ ,  $F_3$  and backcross-plants arising from the cross of  
MS *dicoccum* (Kh)  $\times$  *dicoccoides* (K)

Lines	Pollen fertilities %					
	0	1-25	26-50	51-75	76-100	Total
$F_2$ from MS <i>dicoccum</i> (Kh) $\times$ <i>dicoccoides</i> (K)	10	7	3	10	44	74
Percentage	13.5	9.4	4.1	13.5	59.5	100
[MS <i>dicoccum</i> (Kh) $\times$ <i>dicoccoides</i> (K)] $\times$ <i>dicoccum</i> (Kh)	15	2	5	6	11	39
Percentage	38.5	5.1	12.8	15.4	28.2	100
$F_3$ -plants	10	2	5	4	12	33
$F_2$ -fertile plants $\times$ <i>dicoccum</i> (Kh)	20	6	2	3	6	37
MS <i>dicoccum</i> (Kh) $\times$ $F_2$ -fertile plant	16	4	3	4	6	33
$F_2$ -sterile plants $\times$ <i>dicoccum</i> (Kh)	All pollen sterile					

the pollen restoring factor originating from *dicoccoides* (K) is not one simple gene, but two or more genes.

#### c. Crosses between MS *durum* and Einkorn wheats

Triploid hybrids were obtained from the crosses of MS *durum*  $\times$  *monococcum* or *aegilopolides*. The chromosome behavior at meiosis did not show particularly different features from normal *durum*  $\times$  einkorn wheats. They showed complete sterility on both male and female side, in spite of the vigorous growth. Therefore, no offspring were obtained by backcrossing with the same pollen parents.

d. *Crosses between male-sterile wheats and Dinkel wheats*

Pentaploid hybrids were obtained from the cross between MS *durum* and Dinkel wheats, *vulgare* (e) or *compactum*. Meiosis in the pentaploids proceeds normally, showing a chromosome configuration of  $14\Pi+7\text{I}$  and rarely one of  $13\Pi+9\text{I}$  at MI, like those of the usual pentaploid hybrids between normal Emmer and Dinkel wheats. However, no good pollen grains were produced (cf. usual pentaploids possess 70–80% good pollen: [78]). From the five successive backcrosses with *compactum* or *vulgare*, as seen in Table 4,  $21\Pi$ -plants were obtained in both backcross lines. Without exception they became typical male-sterile plants. Morphologically, they appeared to have the full complement of *compactum* or

TABLE 4

Pedigree of successive backcrosses of MS *durum* with Dinkel wheats, showing chromosome configurations and pollen fertilities

1951	1952	1953	1954
MS <i>durum</i> × <i>vulgare</i> → $14\Pi+7\text{I} \dots 0\%$	→ $\times^v$ $\left[ \begin{array}{l} 1\Pi\Pi+16\Pi+3\text{I} \dots\dots\dots 1.02\% \\ 1\text{IV}+1\Pi\Pi+13\Pi+4\text{I} \dots\dots 0 \end{array} \right]$	→ $\times^v$ $2\text{IV}+16\Pi+2\text{I} \dots\dots 0\%^*$	
MS <i>durum</i> × <i>compactum</i> → $14\Pi+7\text{I} \dots\dots 0$	→ $\times^c$ $\left[ \begin{array}{l} 16\Pi+5\text{I} \\ 16\Pi+4\text{I} \\ 20\Pi+1\text{I} \dots\dots\dots 0.34 \\ 1\Pi\Pi+16\Pi+3\text{I} \end{array} \right]$	→ $\times^c$	→ $\left[ \begin{array}{l} 19\Pi+2\text{I} \dots\dots\dots 0^{**} \\ 18\Pi+4\text{I} \\ 19\Pi+2\text{I} \end{array} \right]$
1955	1956	1957	
$\begin{array}{l} * \times^v \rightarrow \left[ \begin{array}{l} 19\Pi+2\text{I} \dots\dots 0\% \\ 19\Pi+3\text{I} \dots\dots 0 \\ 20\Pi+1\text{I} \dots\dots 0 \\ 19\Pi+2\text{I} \dots\dots 0 \\ 19\Pi+2\text{I} \dots\dots 0 \end{array} \right] \end{array}$	$\begin{array}{l} \times^v \rightarrow \left[ \begin{array}{l} 20\Pi+2\text{I} \dots\dots 0\% \\ 20\Pi+1\text{I} \dots\dots 0 \end{array} \right] \\ \times^v \rightarrow 1\text{IV}+19\Pi \dots\dots 0 \end{array}$	$\begin{array}{l} \times^v \rightarrow 1\text{IV}+18\Pi+2\text{I} \dots\dots 0\% \\ \times^v \rightarrow \left[ \begin{array}{l} 21\Pi \dots\dots\dots 0 \\ 21\Pi \dots\dots\dots 0 \\ 21\Pi \dots\dots\dots 0 \end{array} \right] \end{array}$	
$\begin{array}{l} ** \times^c \rightarrow 20\Pi+1\text{I} \dots\dots 0 \end{array}$	$\begin{array}{l} \times^c \rightarrow \left[ \begin{array}{l} 21\Pi \dots\dots\dots 0 \\ 21\Pi \dots\dots\dots 0 \\ 21\Pi \dots\dots\dots 0 \end{array} \right] \end{array}$	$\begin{array}{l} \times^c \rightarrow \left[ \begin{array}{l} 21\Pi \dots\dots\dots 0 \\ 21\Pi \dots\dots\dots 0 \end{array} \right] \end{array}$	

v: *vulgare*, c: *compactum*.



Fig. 3. Ears of male-sterile Dinkel wheats with *ovata* cytoplasm. a, normal *compactum*. b, MS *compactum*. c, MS *compactum* × Norin 26. d, MS *compactum* × *durum* (R).

*vulgare* (e) genomes (Fig. 3). However, they showed lower plant height and later maturation than normal *compactum* or *vulgare* (e) plants. From the cross MS *durum* × *Spelta* (D), pentaploid seeds were easily obtained, but they shrivelled strikingly, and did not germinate.

MS *compactum* plants with *ovata* cytoplasm were crossed with Norin 26, which belongs to the Dinkel wheats and it has cultivated well in Japan. Plants resulting from this cross grew vigorously, but showed also male sterility. MS *compactum* plants

were also crossed with the pollen of normal *durum* (*R*) and *dicoccoides* (*K*). The resulting pentaploids with *durum* (*R*) showed also complete male sterility and  $14_{II}+7_I$  and  $13_{II}+9_I$ -chromosome configurations at MI like those of MS *durum*  $\times$  *compactum*. They grew very well, and had nearly the same appearance as MS *durum* plants (Fig. 3). However, pentaploids with *dicoccoides* (*K*) possessed very long leaves with pale-green color, and a strikingly low plant height. They died before maturing.

### 3. Additional informations on nucleus exchange in the *Triticum-Aegilops* group

#### a. Placing of *variabilis* genome in *ovata* cytoplasm

Substitution of *variabilis* genome into *ovata* cytoplasm was accomplished after four successive crosses. The pedigree has been presented in Table 5, showing chromosome configurations at MI and pollen fertilities. Various chromosome configurations other than presented in Table 5 were observed; namely,  $1_{III}+12_{II}$

TABLE 5

Survey of chromosome configurations and pollen fertilities in the course of introducing *variabilis* genome into *ovata* cytoplasm.

1951	1952	1953											
<i>Ae. ovata</i> 14 <sub>II</sub> .....96.0% $\xrightarrow{\times v}$ 1 <sub>IV</sub> +1 <sub>III</sub> +4 <sub>II</sub> +13 <sub>I</sub> .....0.2% $\xrightarrow{\times v}$ <table><tr><td>13<sub>II</sub>+8<sub>I</sub>.....2.0%*</td></tr><tr><td>13<sub>II</sub>+8<sub>I</sub>.....2.7 **</td></tr><tr><td>2<sub>III</sub>+15<sub>II</sub>+6<sub>I</sub>.....7.1</td></tr></table>			13 <sub>II</sub> +8 <sub>I</sub> .....2.0%*	13 <sub>II</sub> +8 <sub>I</sub> .....2.7 **	2 <sub>III</sub> +15 <sub>II</sub> +6 <sub>I</sub> .....7.1								
13 <sub>II</sub> +8 <sub>I</sub> .....2.0%*													
13 <sub>II</sub> +8 <sub>I</sub> .....2.7 **													
2 <sub>III</sub> +15 <sub>II</sub> +6 <sub>I</sub> .....7.1													
1954	1955	1956	1957										
* $\times v$ $\rightarrow$ 14 <sub>II</sub> +1 <sub>I</sub> .....38.7%	$\xrightarrow{s}$ 14 <sub>II</sub> ..78.3% $\xrightarrow{s}$ 14 <sub>II</sub> .....0.1% $\xrightarrow{\times v}$ <table><tr><td>14<sub>II</sub> .....92.7%</td></tr><tr><td>14<sub>II</sub> .....77.9</td></tr></table> $\xrightarrow{\times v}$ <table><tr><td>14<sub>II</sub> .....34.7</td></tr><tr><td>14<sub>II</sub> .....96.8</td></tr></table> $\xrightarrow{s}$ 14 <sub>II</sub> .....45.5 $\xrightarrow{\times v}$ <table><tr><td>14<sub>II</sub> .....94.1</td></tr><tr><td>14<sub>II</sub> .....84.0</td></tr></table>	14 <sub>II</sub> .....92.7%	14 <sub>II</sub> .....77.9	14 <sub>II</sub> .....34.7	14 <sub>II</sub> .....96.8	14 <sub>II</sub> .....94.1	14 <sub>II</sub> .....84.0	$\xrightarrow{\times v}$ 14 <sub>II</sub> .....96.2 $\xrightarrow{s}$ 14 <sub>II</sub> .....84.2 $\xrightarrow{\times v}$ 14 <sub>II</sub> .....94.3	$\xrightarrow{\times v}$ 14 <sub>II</sub> .....75.0 $\xrightarrow{\times v}$ <table><tr><td>14<sub>II</sub> .....91.2</td></tr><tr><td>14<sub>II</sub> .....94.5</td></tr></table> $\xrightarrow{\times v}$ 14 <sub>II</sub> +1 <sub>I</sub> ....88.0 $\xrightarrow{\times v}$ <table><tr><td>14<sub>II</sub> .....91.2</td></tr><tr><td>14<sub>II</sub> .....94.5</td></tr></table>	14 <sub>II</sub> .....91.2	14 <sub>II</sub> .....94.5	14 <sub>II</sub> .....91.2	14 <sub>II</sub> .....94.5
14 <sub>II</sub> .....92.7%													
14 <sub>II</sub> .....77.9													
14 <sub>II</sub> .....34.7													
14 <sub>II</sub> .....96.8													
14 <sub>II</sub> .....94.1													
14 <sub>II</sub> .....84.0													
14 <sub>II</sub> .....91.2													
14 <sub>II</sub> .....94.5													
14 <sub>II</sub> .....91.2													
14 <sub>II</sub> .....94.5													
** $\times v$ $\rightarrow$ <table><tr><td>1<sub>IV</sub>+2<sub>III</sub>+8<sub>II</sub>+3<sub>I</sub>.....49.2%</td></tr><tr><td>14<sub>II</sub>+1<sub>I</sub> .....95.1</td></tr></table>	1 <sub>IV</sub> +2 <sub>III</sub> +8 <sub>II</sub> +3 <sub>I</sub> .....49.2%	14 <sub>II</sub> +1 <sub>I</sub> .....95.1											
1 <sub>IV</sub> +2 <sub>III</sub> +8 <sub>II</sub> +3 <sub>I</sub> .....49.2%													
14 <sub>II</sub> +1 <sub>I</sub> .....95.1													
v: <i>variabilis</i> . s: <i>selfing</i> .													

v: *variabilis*, s: selfing.

+ $7_I$ ,  $12_{II}+10_I$ ,  $5_{III}+10_{II}+5_I$ ,  $1_{IV}+1_{III}+11_{II}+5_I$  and  $2_{III}+15_{II}+6_I$  in 1953, and  $1_{IV}+1_{III}+10_{II}+2_I$ ,  $13_{II}+2_I$  and  $1_{IV}+12_{II}$  in 1954. Some  $14_{II}$ -plants in 1955 also showed a  $13_{II}+2_I$ -configuration in the same anthers, and heteromorphic pairing was observed in a few  $14_{II}$ -plants. In 1956  $14_{II}+1_I$ -plants may have arisen from the result of non-disjunction in such an irregular pairing. Most of the  $14_{II}$ -plants showed high pollen fertility.

From these results it was concluded that *ovata* cytoplasm did not bring about a male-sterile condition, when it combined with *variabilis* genome. *ovata* and *variabilis* possess one common genome [71]. Therefore, the unsubstituted segment of the homologous chromosome is possibly still present after five successive backcrosses. This may present an explanation for the varying degree of pollen fertility in the  $14_{II}$ -plants.

#### b. Placing of *Dinkel* genome into the cytoplasm of *ventricosa* and *dicoccoides* (*sp*)

This experiment was carried out in order to obtain a new hexaploid wheat,



having a D-genome from *ventricosa* [31]. To this end, the  $F_1$  hybrids of *ventricosa* ♀ × Emmer wheats ♂, *durum* and *dicoccoides* (*sp.*), and the reciprocal hybrids were crossed with the pollen of Dinkel wheat, *vulgare* (*e.*). The resulting offspring showed a  $21_{II}+7_I$ -configuration at MI, suggesting the formation of unreduced gametes in the  $F_1$  plants. They were backcrossed successively with *vulgare* pollen. From two or three backcrosses, 42-chromosome plants were obtained, showing  $21_{II}$ -configuration, and modifications of it, such as  $20_{II}+2_I$ ,  $1_{IV}+19_{II}$ , and  $1_{IV}+18_{II}+2_I$ . Their fertilities were nearly normal (80–98%). Morphologically, they were found to be Dinkel wheats, except for one or two characters derived from *ventricosa*. No male-sterile condition was noticed in their offspring. One genome of *ventricosa* is homologous with the D-genome of *vulgare* [72]. Accordingly, in the generations obtained from two or three successive backcrosses with *vulgare* pollen, the whole genes of *vulgare* genome seems to be not fully substituted into *ventricosa* cytoplasm.

c. *Restoration of bicornis genome from the amphidiploid between bicornis and squarrosa*

The author attempted substitution of the D-genome and restoration of the *bicornis* genome in the amphidiploid, *bicornis* ♀ × *squarrosa* ♂. Substitution crosses of the amphidiploid ♀ × *squarrosa* ♂ gave only a few kernels, and only one plant, which was weak and died before maturing. On the other hand, from the restoring cross of the amphidiploid ♀ × *bicornis* ♂, healthy plants were obtained, and further backcrosses gave many  $7_{II}$ -plants. Some of them showed a more vigorous growth than normal *bicornis*, and had high fertility [32].

#### 4. Conclusion and discussion

a. *On the interrelationships between genome and cytoplasm concerning male sterility*

As mentioned above, when all 28 chromosomes of the *durum* genome are introduced in *ovata* cytoplasm through successive backcrosses, the resulting  $14_{II}$ -plants became completely male-sterile, without exceptions. In that case, they did not display *ovata* characters any more. This indicated two possibilities; 1) no crossing-over between *ovata* chromosomes with pollen restoring genes and *durum* chromosomes occurred in the course of successive backcrosses, or 2) a semi-homologous chromosome subjected to crossing-over had been eliminated by the time genome substitution occurred. That is, male-sterile condition appears simultaneously with cytological completion of the genome substitution. On this point, the present material differs from the *Epilobium* hybrids, obtained by Michaelis [100], in which pollen-fertile and -sterile plants have been frequently separated in the offspring. The present case rather resembles that described by Correns [15] for *Circium*, and by Rhoades [127] for maize, where the inheritance of male sterility is solely cytoplasmic.

In *Epilobium* hybrids, attempted to introduce the *luteum* nucleus into the cytoplasm of *hirsutum* failed because of the lethality of the third backcross generation. Such a failure of reciprocal substitution has been frequently reported for the hybrids of many plant species. In my experiments, however, the

TABLE 6  
Results of genome exchange among *Aegilops* and *Triticum* species through successive backcrossing,  
in relation to pollen production

Female parents		Pollen providers		Resulting plants	
Species name	Cytoplasm and genome	Species name	Genome	Cytoplasm and genome	Fertility
<i>Ae. ovata</i>	$\alpha^0$ CuCu MoMo	<i>T. durum</i> (R)	AABB	$\alpha^0$ AABB	Male-sterile
MS <i>durum</i> (R)	" AA BB	<i>T. durum</i> (h)	"	" "	"
"	" "	<i>T. dicocum</i> (E)	"	" "	"
"	" "	<i>T. dicocum</i> (Kh)	"	" "	"
"	" "	<i>T. dicoccoides</i> (K)	"	" "	Fertile
"	" "	" (sp)	"	" "	Male-sterile
"	" "	" (st)	"	" "	"
"	" "	<i>T. vulgare</i> (e)	AABBDD	" AABBDD	"
"	" "	<i>T. compactum</i>	"	" "	"
"	" "	Norin 26	"	" "	"
"	" "	<i>Ae. ovata</i>	CuCu MoMo	" CuCu MoMo	Fertile
<i>ovata</i> with <i>durum</i> cytoplasm	$\beta^d$ CuCu MoMo	<i>T. durum</i> (R)	AA BB	$\beta^d$ AA BB	"
<i>T. durum</i> (R)	" AA BB	<i>Ae. ovata</i>	CuCu MoMo	" CuCu MoMo	"
<i>Ae. ovata</i>	$\alpha^0$ CuCu MoMo	<i>Ae. variabilis</i>	CuCu S <sup>v</sup> S <sup>v</sup>	$\alpha^0$ CuCu S <sup>v</sup> S <sup>v</sup>	"
<i>Ae. ventricosa</i>	$\alpha^v$ DD MoMo	<i>T. vulgare</i> (e)	AABBDD	$\alpha^v$ AABBDD	"
<i>Ae. caudata</i>	$\alpha^c$ CC	<i>T. vulgare</i> (e)	"	$\alpha^c$ "	Male-sterile*
<i>T. dicoccoides</i> (sp)	$\beta^{du}$ AA BB	<i>T. vulgare</i> (e)	"	$\beta^{du}$ "	Fertile

\* After Kihara [75]

reciprocal substitutions of *durum* and *ovata* genomes succeeded, though the second backcross offered great difficulty. Therefore, the results might contribute to clarify the problem of male sterility caused by foreign cytoplasm.

The experimental results obtained in this regard are summarized in Table 6, including some additional findings on the nucleus exchange among *Aegilops* and *Triticum*. Other than the data listed in this Table, *T. turgidum*, *dicoccum* (R), *persicum* (s), *orientale*, *polonicum* and *pyramidale* seem to possess the same genome constitution as *durum* (R) concerning the male sterility, because the successive backcrosses with their pollen produced exclusively malesterile plants.

From the several facts mentioned above, it must be concluded that the cytoplasm of *ovata* and *durum* are certainly different. Moreover, the cytoplasm of *Ae. caudata* possessed a property different from that of *T. vulgare*. The cytoplasm of *T. dicoccoides* (sp) does not show any remarkable effect on the genome manifestation of *vulgare*, concerning male sterility. A similar interpretation can be given to the results obtained from the fertile offspring of pentaploid hybrids between Emmer and Dinkel wheats [66, 98]. On the other hand, the *ovata* cytoplasm does not bring about a male-sterile condition in the presence of the genome of *Ae. variabilis*. In the  $F_4$  generation of the hybrid of *ovata* × *variabilis*, Kihara and Matsumoto [77] have obtained male-sterile offspring which segregated in fertile and sterile plants. Therefore, the male-sterile condition does not seem to be based on a cytoplasmic factor.

At first glance, the cytoplasm of different species of the intra-genus *Triticum* or *Aegilops* seemed to have no effect on other genome manifestation each other. However, the cytoplasm of *Ae. ventricosa* does not bring about such effect on the *vulgare* genome. Therefore, the cytoplasm of *ventricosa* and *ovata* are certainly different. Moreover, the *ovata* cytoplasm seems to have properties different from those of the *caudata* cytoplasm, since the degree of pollen degeneration of MS *vulgare* with *caudata* cytoplasm is somewhat lower than that of MS *vulgare* with *ovata* cytoplasm [cf. 81]. Moreover, cytoplasmic difference between *Ae. longissima* and *Ae. Aucheri* have been found by Kihara [75]. Based on these findings, it may be said that some distinct properties are present in different species of the intra-genus *Aegilops*.

It is well known that a plasmon can change under the influence of a foreign genome. Michaelis [100, 102] showed that when *hirsutum* with *luteum* cytoplasm was crossed with *luteum* pollen, the resulting  $\lambda 8hl$  plants (the number indicates the number of backcrosses) had on the average 20.49% fertile pollen, while the reciprocal  $\lambda 0hl$  had 28.99% fertile pollen. He postulated that during the seven generations of outcrossing to *hirsutum*, it has become changed in such a way as to inhibit pollen fertility more strongly, and that this fact is regarded as a gradual approximation of *luteum* cytoplasm toward *hirsutum* cytoplasm due to the *hirsutum* genome. Moreover, Michaelis [105, 106] has observed numerous alterations of the plasmon, and postulated that they are very probable caused by a sorting out of several cytoplasmic constituents within the plant. He explained that these alterations are, through selection processes within the individual, directed toward an elimination of developmental disturbances. Ono [118, 119] reported that in the hybrids *Paraixeris denticulata* and *Crepidiastrum platy-*



*phyllum* the fertility was much restored in  $F_4$ . He assumed, that the alteration of cytoplasm under influence of a foreign nucleus will take place gradually, both in somatic and in germ cells. Schwemmle et al. [137] reported that in *Oenothera* a gradual disappearance of matrocliny took place under the influence of a certain gene complex, but in other complexes the maternal influence does not diminish. Michaelis has also assumed from his study on *Epilobium* hybrids, that internal factors, like genotype, are more important for the processes, which alter the plasmon, than external conditions. In *Paramecium*, a spontaneous nuclear mutation involving loss of Kappa particles has been reported [51], and also several kinds of mutations in the Kappa particle itself [20, 50, 51].

It is remarkable that the *ovata* cytoplasm has been maintained unchanged through 11 generations of backcrosses. Further, the special property affecting pollen production has not undergone any change in over 30 years, because the *Aegilotriticum*, used in my experiment, was synthesized in 1926 [155]. There are no remarkable differences in the degree of pollen production between the MS *durum* derived from the *Aegilotriticum* and from a pure *ovata* plants. No fertile plants appear in the offspring. Moreover, the cytoplasm has not been altered physiologically and morphologically. Therefore, gradual changes, as found in *Epilobium* or other plants, have not been recognized in the present material. In other words, the cytoplasm of *Aegilops* is very constant, and cannot be altered by foreign *Triticum* genome. If the specific plasmon would be particulate, as proposed in the theory of particulate plasmagenes, the particles may not consist of different entities, but represent one type. However, their action seems to be multiple, as will be described later. The particles had to be so abundant numerically that they would not be lost during somatic and meiotic cell division.

b. *Conclusion on, and discussion of the factor, which restor fertility*

As mentioned above, *dicoccoides* (*K*) possesses some particular nuclear genes affecting pollen restoration in MS Emmer wheat with *ovata* cytoplasm. Since the  $F_1$  hybrids of MS Emmer  $\times$  *dicoccoides* (*K*) become fertile, the nuclear gene or genes must be dominant. The  $F_2$  and  $BC_1$  generation produce many intermediate individuals with various degree of pollen fertility. Therefore, two or more alleles seem to be involved in pollen restoration. From the facts that some sterile plants appear in the  $F_2$  offspring, it seems to follow that the restoring genes do not act by destroying completely the sterility-causing cytoplasmic element or to transform it into normal fertile cytoplasm, but act by inhibiting temporarily the sterility-causing action of the cytoplasmic element.

It is important to say that the plants with 14 bivalents of the *durum* genome and some extra-chromosomes of *ovata* genome produce good pollen grains in various amount depending on the extra-chromosomes and on environmental conditions. As described in a preceding paper [37], some  $14_{II}+1_I$ -plants showed nearly normal pollen fertility. In other words, all pollen grains (14- or 15-chromosome pollen) are functional irrespective of the presence or absence of an extra-chromosome in its microspores. Therefore, it may be concluded that the fertility restoring extra-chromosome exerts its effect only in the sporophyte—not in the

male gametophyte. However, a following question remains yet, that the extra-chromosome may exert its effect in the pollen mother cells, before the chromosome reduction take place. Gabelman [47] concluded in his study of cytoplasmic partial male-sterility in maize that pollen degeneration was due to a particulate factor in cytoplasm and that the presence of one or none of these particles in a microspore resulted in its failure to develop into mature pollen. That is, its action is gametophytic. However, in the case of cytoplasmically induced male-sterility of *Allium* and *Beta*, conspicuous tapetum abnormalities have been observed [4, 150]. It may be said that these cytoplasmic factors possess a sporophytic

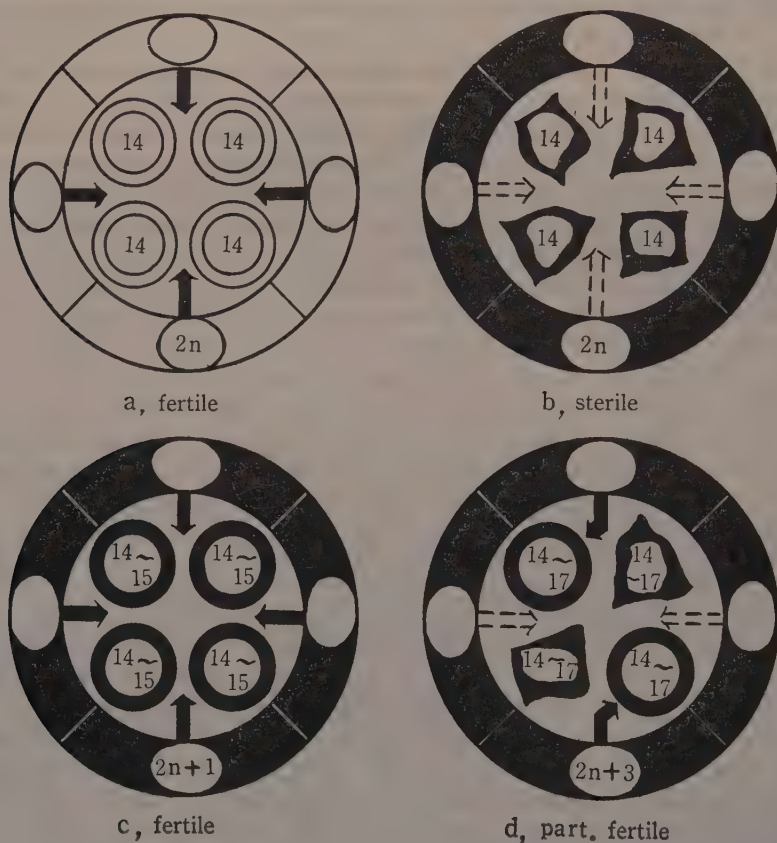


Fig. 4. Diagrams showing the interrelationships between cytoplasm and chromosomal factors regarding pollen production. The outer large zone represents tapetum cells, the inner circles pollen grains. Small circles containing ciphers indicate the chromosome numbers of the nuclei. Fertile pollen grains are represented by regular round circles and abortive pollen is indicated by irregular contours. *ovata* cytoplasm is black. a, normal *durum*. b, male-sterile *durum* with *ovata* cytoplasm. c,  $14_{II}+1_{I-}$ -plants. d,  $14_{II}+X_{1-}$ -plants, representing only one case, partially fertile.  $\longrightarrow$  represents normal supply of nutrition essential for microspore development, while  $\dashrightarrow$  means insufficient supply.

action. In the male-sterile wheat plants, pollen degeneration occurs shortly after the liberation from the tetrads without any tapetum abnormalities. It is quite possible that the fertility-restoring chromosome in question is closely related to the production or transmission of nutritive factors [37]. Thus, the mechanism of pollen-restoring action of an extra-chromosome from the *ovata* genome may be interpreted tentatively by schematic diagrams, as shown in Fig. 4. In male-sterile anthers (Fig. 4b), owing to disharmony between *ovata* cytoplasm and *durum* genome in tapetal cells (or other somatic cells), the normal supply of nutrition from tapetal cells into microspores may be strikingly disturbed. Such poor nutrition seems to lead to an abnormal pollen mitosis, as will be described in next section. In the  $14_{II}+1_I$ -plants in which the extra-chromosome possess a certain action to inhibit completely the specific property of *ovata* cytoplasm (Fig. 4c), a normal supply of nutrition takes place, inspite of the combination of *durum* genome and *ovata* cytoplasm in the pollen grains. In partially fertile plants (Fig. 4d), extra-chromosomes derived from *ovata* are present in somatic cells. Although they may possess a certain activity in producing essential nutrition, their action seems to be partially disturbed owing to the unbalanced genome constitution in the somatic tissue. Accordingly, some of microspores can not take up nutrition sufficient for normal development, especially the microspores possessing more extra-chromosomes. The partial pollen fertility of  $14_{II}+x_1$ -plants in the substitution lines is based on the combination of three possible factors; 1) a certain cytoplasmic element derived from *ovata* plants, 2) a pollen-restoring factor of extra-chromosomes from *ovata* plants, 3) meiotic irregularities, brought about by univalents in the microsporocytes.

In *Paramecium*, Sonneborn [141, 142] discovered an interesting cytoplasmic inheritance, in that cells which do not contain Kappa particles in the cytoplasm are sensitive, and are killed by coming in contact with paramecin produced by a killer. But, the killer strain carries the dominant gene *K* in the nucleus, and is not influenced by the paramecin. In my experiments, *ovata* plants were not affected by the special *ovata* cytoplasm which leads to a male-sterile condition, when it was combined with Emmer or Dinkel wheats. Recently, Cameron [8] reported, in the hybrid between *Nicotiana tabacum* and *N. plumbaginifolia*, that *kl* (pollen killer) locus has no deleterious effect on pollen of *plumbaginifolia* itself, but all microgametophytes with a complete complement of *tabacum* chromosomes are abortive after meiosis. The killing effect is apparently restricted to the PMC gametes in which the *kl* chromosome is present.

As seen in the course of genome substitution, the pollen-restoring action by chromosomes from *Ae. ovata* seems to be present in two more chromosomes. As mentioned above, however, the special factor as seen in *ovata* cytoplasm has not been produced in the *durum* cytoplasm which maintained through several generations under the influence of *ovata* genome. Therefore, the hypothesis of gene-produced plasmagene postulated by Wright [164] and Spiegelman [148] does not apply in the present case. It is also clearly different from a delayed nuclear effect resulting from the transmission of gene products, as was postulated by Lehmann [87] in reciprocally different *Epilobium* hybrids, or the so-called genotypic predetermination [140]. It is also different from a dauermodification, as found



occasionally in *Phaseolus* [52, 138, 139], in tomato [90], in tobacco [53], and other plant species. From these findings, the interrelation between genome and plasmon may be tentatively described as follows; the specific cytoplasmic factor in *ovata* plants did not exist before a phylogenetic separation between *Aegilops* and *Triticum* group took place from an unknown form. After the separation, such factors were produced through cytoplasmic mutation in *ovata* cytoplasm. As assumed by Woods [160] and duBuy and Woods [22], the cytoplasmic factor might be transferred by mitochondria, or, as assumed by Altenburg [2, 3] by symbionts. Or normal cell proteins might become such a factor, as postulated by Darlington [18]. Moreover, it is possible that the cytoplasmic elements come from Feulgen positive bodies present in cytoplasm which is produced as a result of irregular hybrid meiosis. These bodies also occasionally occur in the meiotic prophase [146, 13], and rarely in somatic cells [64]. In that connection, some environmental agents might bring their formation about. The effect of such agents is shown in the cytoplasmic alteration, represented by "irregulare" in *Epilobium*, which arises either after treatment with radioactive isotopes, or spontaneously in certain *hirsutum* hybrids [106]. The possibility that an environmental factor plays a role is supported by the fact that cytoplasmic mutant in yeast can be induced with acriflavin treatment [29]. Rhoades [129] discovered an interesting finding in *Zea mays*, namely, that plants, homozygous for *ij*, produced frequently a male sterile condition, and it inherited cytoplasmically by later generations. Accordingly, *ovata* genome, having a similar gene complex, might induce the cytoplasmic factor in question, and after that such particular gene or gene complex may lose this ability by mutation. The pollen-restoring genes present in the *dicoccoides* (*K*) nucleus might have been produced independently of the cytoplasmic factor.

#### IV. CYTOLOGICAL AND BIOCHEMICAL INVESTIGATIONS ON POLLEN DEGENERATION IN MALE-STERILE PLANTS

##### 1. *Cyto-histological investigations of pollen degeneration*

###### a. *Pollen grains in mature anthers of male-sterile plants*

At the time of flowering, anther smears from MS plants show numerous pollen grains, characterized generally by smaller size than those of normal plants. Various types of degeneration have been observed, namely: some had only shrivelled cell walls and no contents, others contained one or two nuclei with a small amount of cytoplasm, and, in a few cells, three not fully differentiated nuclei. As shown in Table 7, these abortive pollen grains have been classified in five grades [38], according to Kihara's classification [71]. Some of pollen grains possess micro-chromatin bodies. Polynucleated microspores with four or five nuclei are rarely observed. These facts indicate that the division of pollen nuclei did not proceed normally [38].

TABLE 7  
Frequency percentage of pollen grains classed in five grades

Strains	Pollen classes*				
	I	II	III	IV	V
MS <i>durum</i> (R)	0	2.0	20.1	43.0	34.9
MS <i>dicoccum</i> (Kh)	0	0.6	15.3	28.1	56.0
<i>T. durum</i> (R)	96.0	0.4	0.6	0.9	1.9
<i>T. dicoccum</i> (Kh)	94.1	1.1	2.1	0.8	1.9
<i>Ae. ovata</i>	96.0	0.2	0.2	0.9	2.7
MS <i>dicoccum</i> (Kh) × <i>dicoccoides</i> (K)	97.1	0.4	0.7	0.4	1.4
14 <sub>II</sub> +1 <sub>I</sub> -plant in SB line	92.0	0.6	2.6	1.4	3.4

\* Pollen classes: I, good pollen. II, pollen grains with three not fully differentiated nuclei. III, two nucleate pollen. IV, one nucleate pollen. V, empty pollen.

b. *Young pollen grains shortly after the liberation from tetrads*

Germ-pore formation of microspores in MS *durum* begins soon after the liberation from tetrads, together with the thickening of the cell wall. Following the germ-pore formation, the nucleus moves to the opposite side of the germ-pore, and a large vacuole appeared in the center of the cell. No cytological aberrations occurs until that stage. Afterward, however, the cytoplasm and the nucleus in some cells disappear and the microspores become empty pollen grains. Some cells show a nearly normal pollen-mitosis figure, with a considerable amount

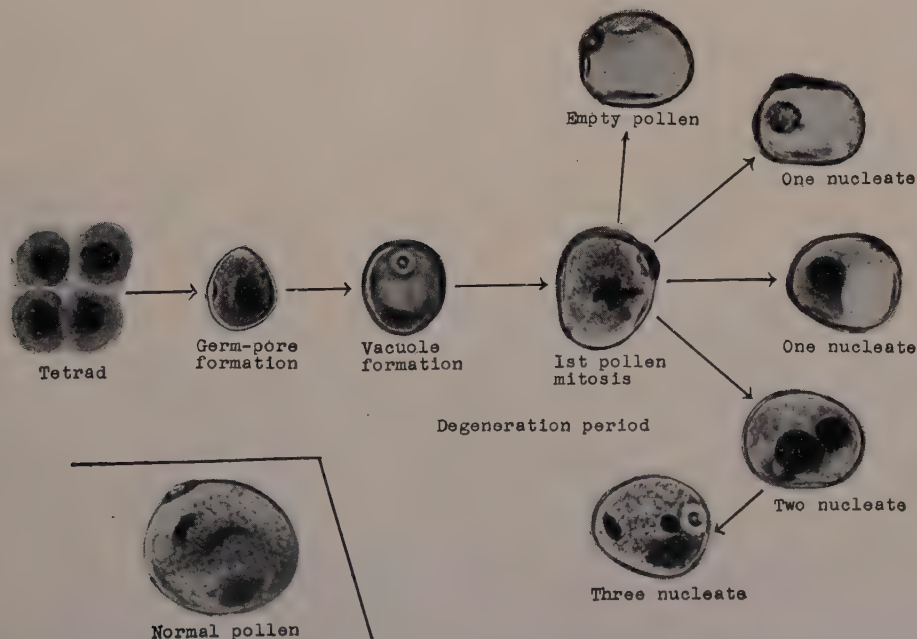


Fig. 5. Abortive development of microspores in male-sterile *durum* plants. ca.  $\times 220$ .

of cytoplasm, but the next pollen mitosis seems to be disturbed [38].

From the above two examinations, (1) and (2), it seems reasonable to conclude that pollen degeneration of the male-sterile plants begins in most case during the first pollen mitosis (Fig. 5).

Rhoades [127] has reported in maize a marked difference in size, shape and number of cytoplasmic elements in microspores, fixed in Benda's fluid and stained with hematoxylin. In wheats, however, there was no pronounced difference between male-sterile and normal plants in regard to cytoplasmic entities of anther tissues and microspores. Especially, there was no indication of Feulgen positive particles as seen in *Paramecium* [123, 124].

c. *Pollen grains in the offspring derived from male-sterile plants × dicoccoides (K)*

As mentioned in previous section, the hybrids between MS *dicoccum* (Kh) and *dicoccoides* (K) produced pollen grains as many normal pollen grains as normal plants (Table 7). In the next generation, obtained by selfing and backcrossing with *dicoccum* (Kh) pollen, they segregate in individuals, having various pollen fertilities, ranging from 0 to 96%. They were divided into five classes according to the degree of pollen fertility. As seen in Table 8, there were no great

TABLE 8  
Pollen analysis in the offspring obtained from the crosses  
MS *dicoccum* (Kh) × *dicoccoides* (K)

Degree of fertility %	Pollen classes					Total
	I	II	III	IV	V	
0	0	1.2	14.3	31.6	52.9	100
	0	1.4	11.3	21.2	66.1	100
1- 25	8.7	12.6	34.1	30.1	14.5	100
	1.3	6.1	19.8	22.3	50.5	100
26- 50	34.1	19.9	22.0	15.5	8.5	100
	40.6	13.9	16.1	14.5	14.9	100
51- 75	65.3	9.2	11.1	6.7	7.7	100
	65.0	18.0	8.5	4.3	4.2	100
76-100	88.3	2.6	2.5	1.9	4.7	100
	84.3	3.3	3.6	2.8	6.0	100

F<sub>2</sub>: gothic, BC<sub>1</sub>: regular letter.

difference between F<sub>2</sub> and BC<sub>1</sub> groups concerning the percentage in each classes.

d. *Histological aspects of sterile anthers* [38]

The tapetal cells of young anthers, as in meiotic prophase, possess large nuclei embedded in dense cytoplasm. Following the microspore development, the cytoplasm of tapetal cells decreased. The cells formed a thin lining, in which individual cells were tangentially stretched. There is no indication of abnormal tapetum hypertrophy or plasmodium formation, as has been observed in sterile anthers of *Beta vulgaris* [4], *Epilobium* [100], and *Allium Cepa* [150].

Normal anthers always consist of four pollen sacs (loculi), although they are destined to fuse with each other to form two sacs in the mature anthers. In



some sterile anthers, it was found that they sometimes consist of three pollen sacs. This malformation has even been observed in young anthers, at the meiotic prophase. In some cases, malformations were limited to only a part of anther.

## 2. *Free amino acids and sugars in the sterile anthers*

### a. *Free amino acids and amides*

Free amino acids in anthers were examined by means of ascending paper chromatography, comparing male-sterile and normal fertile anthers [36, 44]. Anthers used for the survey were mainly taken at the mitotic stage of the pollen grains in which pollen degeneration occurs cytologically. The paper chromatogram revealed the presence more than fifteen different ninhydrin positive substances, among which proline and asparagine showed a remarkable difference between male-sterile and normal fertile anthers. Normal *durum* anthers showed a large spot of proline, while male-sterile anthers gave no proline spot. On the other hand, the asparagine spot of male-sterile anthers was larger than that of normal *durum* anthers. A similar difference regarding proline and asparagine spots was also found between the anthers of cytoplasmic male-sterile maize and those of normal maize plants [36]. Such a difference between proline and asparagine could possibly be considered to be connected with the pollen degeneration, because it does not occur until just before the period, in which pollen becomes disorganized in the male-sterile anthers.

Furthermore, amino acids in the mature flag leaves of MS *durum* plants were examined by means of paper chromatography [44], showing that the asparagine spot of the MS *durum* leaves was larger and the proline spot smaller than that of normal *durum* plants. Furthermore, the anthers and leaves of dark-grown *durum* plants were surveyed for asparagine and proline. As already described, dark-grown *durum* plants show nearly the same pollen abortion as male-sterile anthers. The results showed almost the same relationship between asparagine and proline spots as that of male-sterile plants. An attempt to supply a proline solution to the young spikes of male-sterile plants was made in only few trials: no effect on microspore development was found. More detailed and extensive experiments are needed on this point.

### b. *Sugars in anthers and pollen degeneration*

A survey of sugars in the anthers of male-sterile wheat plants was made by means of one-dimensional paper chromatography [46]. Normal anthers from *durum* wheat gave three spots, in which one small spot represented sucrose and two large spots fructose and glucose, respectively. Only two spots appeared in MS *durum* anthers, with no spot in the sucrose position. Thus, it may be said that sucrose content per anther is decreased in male sterile plants, as compared to that of normal ones.

When the stalked young spikes of MS *durum* plants were cultured in sugar solution in daylight, they grew better than those in the medium without sugar. No good pollen was produced, though the addition of 5% sucrose to the medium was found to be efficacious to some extent in improving the microspore development. From these results it may be assumed that pollen degeneration in

cytoplasmic male-sterile wheats is caused not solely by the deficiency of sucrose in the anthers, but also by other still unknown factors [46].

### 3. Discussion

It is well known that pollen abortion in a hybrid between plants with different chromosome numbers is principally the result of meiotic irregularities. In cytoplasmic male-sterile plants, however, meiosis of PMC appears to be normal. Based on the fact, that microspore development up to germ-pore formation proceeds normally, and that most of the abortive pollen grains in mature anthers possess one nucleus, it may be concluded that the degeneration of pollen grains begins at about the first division of the pollen nucleus. It has been frequently observed in other cytoplasmic male-sterile plants that pollen abortion is associated with abnormalities in the periplasmodium or in the cellular tapetum [4, 54, 96, 100, 132, 150]. In the present male-sterile wheat, however, progressive degeneration of nuclei and cytoplasm in the tapetum cells proceeded normally, like that found in normal *durum* plants. So that it is difficult to ascribe to the tapetum any direct harmful influence upon the developing microspores. The factor, causing pollen abortion must be partly attributed to some other parts than the tapetum or the anthers. This argument is strengthened by the fact that malformed anthers are occasionally produced [38] and that male-sterile plants grown in a greenhouse in winter produced very small anthers [35], suggesting that environmental condition have a marked influence upon anther degeneration [34].

From the above findings it may be concluded that the male-sterile condition results mainly from the failure of nutrient production in pollen grains, or of their transport through the tapetum, during the period immediately following germ-pore formation, or from the failure of their biosynthesis in other vegetative parts.

The development of microspores, according to La Cour [85], Cooper [13], Sparrow and Hammond [146], requires a large amount of nutrition obtained through the tapetum cells, which is essential for the growth and differentiation of the microspores. Studies on excised anther cultures, including autoradiographic analysis, have shown that there is a critical period concerning microspore development [93, 147, 151, 152, 153]. Fukasawa [36] has reported the disappearance of proline and the remarkable accumulation of asparagine in the anthers of cytoplasmic male-sterile wheats and maize in the course of pollen degeneration. Similar findings were reported by Khoo and Stinson [65] for cytoplasmic male-sterile maize. They observed also difference in content of alanine, which also increased in normal mature anthers. However, the accumulation begins later and takes place at a slower rate than in sterile anthers. It is also reported that the free amino acid content of restored sterile anthers was the same as that in normal plants without the sterile cytoplasm [60]. Fukasawa [38] reported further that a similar relationship between proline and asparagine also exists in the mature flag leaves of MS *durum* plants, as well as in both anthers and leaves of dark-grown *durum* plants. Experiments, in which male-sterile spikes were cultured in proline solution, or such solution was injected in young sheath, did not show normal pollen production. Iwanami [56] reported that an increase in starch was

found in the pollen grains of *Impatiens*, in a medium with 5% sucrose. However, when MS *durum* plants were cultured, the addition of sucrose did not result in the production of good pollen grains, though some signs of progressive pollen development were observed, in cytological investigations [46]. At any rate, such a disturbance on the male side could be attributed to certain elements which have self-reproducing capacity in the cytoplasm. It seems to me very important to know whether such a factor would be related closely with the cytochrome system (mitochondria), as found in *Neurospora* by Mitchell [113].

## V. CHARACTERISTIC APPEARANCE CAUSED BY FOREIGN CYTOPLASM

### 1. *Conspicuous abnormalities of vegetative growth*

When the genome were replaced in their own cytoplasm from the hybrid, the resulting plants appear to be normal plants, morphologically and physiologically, as well as in regard to pollen production. Substitution of the genome, however, gives some remarkable physiological dissimilarities from the normal plants with its own cytoplasm. The appearance of such plants seems to be due to the influence of foreign cytoplasm on the manifestation of the genome, because they do not show any segregation among the progeny, and the inheritance is only through the maternal plants.

#### a. *Retardation of vegetative growth (indicated by heading date)*

Heading of *T. durum* begins more than two weeks later than that of *Ae. ovata*. In the course of introducing the *durum* genome into *ovata* cytoplasm, it was noticed, that the heading of the most plants with a small number of extra-chromosome (for example,  $14_{II}+1_I$ ) occurred later than that of the plants with a large chromosome number. Completely substituted  $14_{II}$ -plants matured the latest. They possessed smaller kernels than normal *durum* plants. Germination



Fig. 6. Normal and male-sterile *durum* plants (March 28, 1956).



is one or two days later than that of normal kernels. Subsequent vegetative growth is also slow (Fig. 6), and the heading is delayed two weeks beyond that of normal *durum* plants. The degree of retardation in heading is dependent greatly upon the date of sowing. That is, the individuals grown from the later sowing show a more striking retardation of vegetative growth than those of the earlier sowing [41].

The degree of retardation in MS Emmer wheats varies greatly, when different species were used as pollen parents. When *dicoccum* (E) or *dicoccum* (R) plants, which possess a later maturing habit than *durum* (R), are used as pollinators in the crosses with MS *durum* plants, the resulting plants show a greater retardation in vegetative growth than the MS *durum* plants. By contrast, when the pollen of *dicoccum* (Kh) plants, whose heading is very much earlier than normal *durum* (R) plants, are used with MS *durum* plants, the hybrid shows earlier heading than the MS *durum* plants. If, however, the date of heading in this strain is compared with that of normal *dicoccum* (Kh) plants, a considerable degree of retardation is found [41].

The  $\beta^d$  *ovata* plants do not show male-sterility, but normal fertility is found in both male and female side. Germination is normal. After that, however, a slight degree of retardation in vegetative growth occurs (Fig. 7). For example,



Fig. 7. Normal *ovata* (left) and  $\beta^d$  *ovata* plants (right) (March 28, 1958).

the heading of  $\beta^d$  *ovata* plants lags for more than one week as compared with that of normal *ovata* plants or of restored *ovata* plants [41].

b. *Reduction in plant height*

*T. durum* (R) is a very tall plant, 150 cm. in height, while *Ae. ovata* is a dwarf, about 50 cm. high. The  $F_1$  hybrids between them show an intermediate plant height. In the course of introducing *durum* genome into *ovata* cytoplasm, their plant height varies. Completely substituted  $14_{II}$ -plants become uniform in

height, but have a considerably lower culm than that of normal *durum* plants. It is noticeable that the degree of this different plant height between normal and MS *durum* plants varies with the season of sowing. That is, the difference between these two strains, when sown early is smaller than that following a later sowing. When the pollen of 14 $\Pi$ +1 $\Gamma$ -plants (a substitution strain) is applied to the stigmata of normal *durum* plants, the resulting 14 $\Pi$ -plants show a normal plant height as well as normal pollen production [41].

## 2. Chlorophyll variegation and abnormal stomata

### a. Chlorophyll variegation

A conspicuous chlorophyll variegation appears in the leaves of MS *durum* plants during the winter season, showing a virus-like variegation. It is, however, not transmitted to normal *durum* leaves. The examination of the epidermal cells does not show an X-body as seen in the leaves infected by virus. The variegation is not observed at the beginning of growth after germination, and also does not become obvious after the vigorous growth during the early summer season. The expression of chlorophyll variegation varies clearly with the different strains used as pollinators, and with the various chromosome combinations of *ovata* genome which coexist with complete *durum* genome.

In order to determine to what extent the chlorophyll content was reduced, paper chromatographic analysis was adopted for the variegated leaves of MS *durum* plants and the green leaves of normal *durum* plants [45]. The R<sub>f</sub>-values of chlorophyll-a and -b became greater in proportion to the increase in sample quantity. Thus, it was found that the concentration of chlorophyll-a and -b of variegated leaves was reduced to respectively about 70% and 60% of that of normal green leaves.

### b. Abnormal stomata in the variegated leaves of male-sterile plants [42]

The variegated leaves show a somewhat irregular surface possessing a smaller leaf size than that of the normal *durum* plants. Conspicuous malformed or non-differentiated stomata are frequently observed in the variegated leaves. Various abnormal stomata were classified in six types, following two criteria: 1) the degree of differentiation and malformation of guard cells and its nuclei, 2) the degree of malformation or deficiency of subsidiary cells. Abnormal stomata with strikingly reduced guard cells did not seem to function normally.

Histological observation of abnormal stomata was made by cross-sectioning of the variegated leaves. Palisade cells in the variegated portion are round and arranged somewhat irregularly. Chloroplasts in such cells are pale yellow, resembling the modified plastids infected by virus. This material may represent good material for the survey of modified mitochondria, as reported by Woods and duBuy [162, 163]. The inner air-spaces of abnormal stomata are, generally, small and irregular in comparison to those of normal *durum* plants.

Normal *durum* plants possess a larger number of stomata in longitudinal than in transverse direction. On the other hand, in MS *durum* plants more stomata were counted in transverse than in longitudinal direction. There is no change in stomatal frequency per unit area. The frequency of abnormal stomata

was investigated in longitudinal and in transverse directions. Abnormalities in the extremely variegated portion reached a high of 38%, but the mean values in the whole leaves were 12% and 9% in longitudinal and in transverse directions, respectively. As mentioned above, mature leaves (flag leaves) of MS *durum* plants do not have abnormal stomata. The winter leaves of MS *dicoccum* (Kh) plants, in which chlorophyll variegation does not appear, did not have abnormal stomata.

The length of guard cells and subsidiary cells in the variegated leaves of MS *durum* plants was measured by the use of preparations in which abundant abnormal stomata were observed. There is an extreme variability in the cell length of guard cells, ranging from 9.4–75.4  $\mu$ , and also in the length of subsidiary cells, ranging from 28.3 to 80.1  $\mu$ . F-tests between normal and MS *durum* plants showed significant difference in both guard cell and subsidiary cells. Cell width of subsidiary cells in variegated leaves was measured. No difference in mean width between the leaves of normal and male-sterile plants was found, though a high variance was found in male-sterile plants.

The measurement results of nuclear length of guard cells and subsidiary cells showed that nuclear length of guard cells in male sterile plants varied widely, ranging from 4.7 to 51.2  $\mu$ . F-tests showed a clearly significant difference between normal and male-sterile plants. It was also found that most of the nuclei in abnormal stomata are decreased in the length. On the other hand, nuclei in subsidiary cells of abnormal stomata do not show any remarkable change in appearance.

The correlation was examined between nuclear length and cell length in abnormal stomata of MS *durum* plants. The examination gave an interesting result, showing that the correlation coefficient was plus 0.9137. This means that longer guard cells possess longer nuclei.

### 3. Discussion

It is remarkable that the cytoplasmic male-sterile factor in *Zea mays* apparently has no noticeable effect except with reference to pollen sterility. However, a striking flower malformation has been found in cytoplasmic male-sterile tobacco [12] and in *Solanum* hybrids [83, 84]. *Epilobium hirsutum* plants with *luteum* cytoplasm were pollen sterile, but showed no inhibition of vegetative growth [101]. However, plants with *hirsutum*-Jena cytoplasm were stunted or dwarfed [103, 104]. As described above, four conspicuous characters are most remarkable in MS *durum* plants; namely, 1) retardation of vegetative growth, 2) reduction in plant height, 3) chlorophyll variegation in winter leaves, 4) abnormalities of stomata in variegated leaves. These altered characters do not show any segregation among the progeny, and the inheritance is only through the maternal plant.  $\beta^d$  *ovata* plants have only a somewhat retarded heading date than normal *ovata* plants, but they possess a normal pollen fertility. No chlorophyll variegation appears in  $\beta^1$  *ovata* plants. Michaelis [108, 109] assumed that slight disharmonies between nucleus and cytoplasm may stimulate heterosis. In the present material, however, such a positive heterosis has not been observed. Jones [58] also mentioned that the cytoplasm has no effect on heterosis in male-sterile maize.



In *Epilobium hirsutum* hybrids with Jena plasmon various degree of developmental disturbances were observed [103, 104]. They could be altered by such experimental means as heteroauxin treatment, low temperature, or short day condition [131]. However, artificial restoration of pollen fertility in male-sterile plants seems to be more difficult than removal of vegetative disturbances, because the developmental norm in the generative system is quite different from that in vegetative growth.

The MS *durum* plants show a conspicuous chlorophyll variegation in winter leaves [41]. The appearance of chlorophyll variegation varies obviously with the different species used as pollinators, and with the various chromosome combinations of *ovata* genome which coexist with *durum* genome. The occurrence of chlorophyll variegation may be interpreted by the plastogene theory [55], or the mutated chondriogene theory, in which the mitochondria can mutate only in the presence of a mutation-allowing gene [22]. In the present case, it may be considered that the expression of chlorophyll variegation is based on the combination of three factors, 1) *ovata* cytoplasm, 2) low temperature, and 3) nuclear genes which might be compared with the plasmon-sensitive gene (cf. 111, 128).

It is well known that stomatal frequency is changeable by environmental conditions. Allsop [1] reported that in *Marsilea* an increase of the concentration of sugars in the medium caused an increase in the stomatal frequency. Douwes [21] reported results of measurements of the stomatal number of a unit surface among hybrids of the cross *Epilobium hirsutum* Jena  $\times$  Algiers. The offspring, containing Jena cytoplasm, are crippled, and possess a low stomatal number. In the present material, however, stomatal frequency per unit area was not altered, but the stomatal distribution in longitudinal and transverse directions has undergone a change.

Bartels [5] reported abnormal epidermis formation by a plasmon-change in *Epilobium*. In the present case, also, the variegated leaves showed a striking malformation of stomatal cells [42, 43]. Cross-sections of variegated leaves revealed that palisade cells in the variegated area were rather round and arranged irregularly. Chloroplasts in such cells were pale yellow, and the inner spaces of abnormal stomata were, in general, small and irregular. The same was found in *Nepeta* variegations by Woods and duBuy [163].

In mature leaves of MS *durum* plants, in which chlorophyll variegation has not been observed up to this time, abnormal stomata have not been observed either. Also, MS *dicoccum* (Kh) plants, in which chlorophyll variegation did not appear in the winter leaves, did not show abnormal stomata either. Further, the frequency of abnormality varied much more in the variegated portion than in the green area of the same leaf. These findings suggest that the formation of abnormal stomata might be closely related with chlorophyll variegation. Stomata in leaves, however, form at an early developmental stage, at which chlorophyll formation has not yet begun. Therefore, it may be possible that the abnormal stomata are not induced directly by chlorophyll abnormalities. Woods and duBuy [161] postulated, from a study on variegated *Hosta*, that high temperature seems to favor multiplication of one chondriogene, low temperature that of the other. In the present material, as abnormal stomata and chlorophyll

variegation occur only at low temperature, the causal factor must be temperature-sensitive. According to duBuy's opinion, a mitochondrial mutation might be primary, causing abnormal metabolism, leading to abnormal development of stomata, on the one hand, and, on the other, to formation of abnormal plastids in parenchyma cells. This would lead to further abnormalities in these cells. Moreover, as described already, since subsidiary cells of all abnormal stomata are malformed or missing, and since the guard cells of some abnormal stomata do not differentiate, these stomata may not be functional. Therefore, abnormal stomata would accelerate the occurrence of variegations and the formation of abnormal plastids.

## VI. CROSS-COMPATIBILITY AND EMBRYO DEVELOPMENT IN RELATION TO FOREIGN CYTOPLASM

### 1. Seed-setting in male-sterile plants and its embryological aspects

#### a. Seed-setting of *MS durum* plants [40]

The seed-setting of normal *durum* plants was 95% in 1956; while that of *MS durum* × normal *durum* was 98%. The average percentage for seven years from 1950 to 1956 in *MS durum* was 93.3%. From the  $\chi^2$ -test, these values did not give a significant difference from that of normal *durum*. Furthermore,  $\beta^d$  *ovata* plants were pollinated with normal *ovata* pollen, resulting in the same crossability as that between normal *ovata* plants themselves. These results show that foreign cytoplasm does not have any harmful influence on crossability.

#### b. Embryo development of male-sterile *Emmer* wheats

Seed germination of *MS durum* plants was delayed one or two days, compared to that of normal *durum* plants. Also, further growth is slow. This retardation of growth may be started during embryo development. With this point in mind, comparative observations of embryo development between *MS durum* and normal *durum* plants were made.

Ovules taken just before flowering were cut longitudinally,

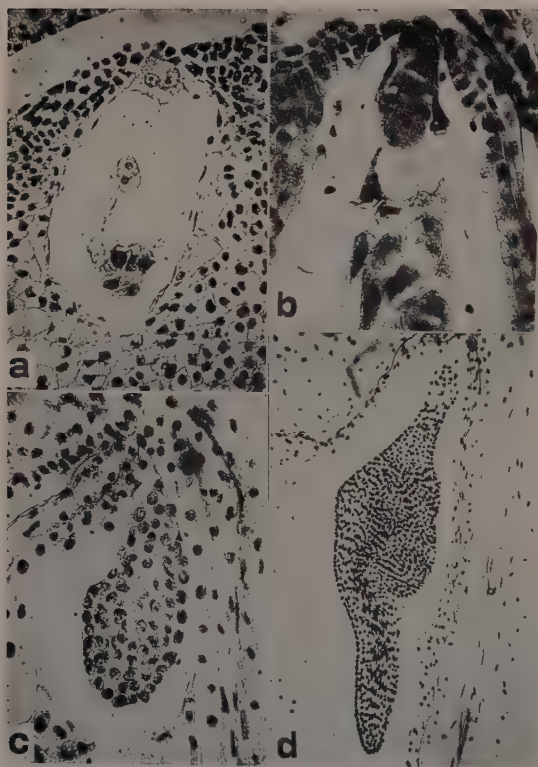


Fig. 8. Embryo development of male-sterile *durum*. a, embryo-sac.  $\times 190$ . b, proembryo 2 days old.  $\times 200$ . c, 6 days.  $\times 140$ . d, 10 days.  $\times 57$ .

and examined morphologically. As shown in Fig. 8, the embryosac of MS *durum* plants possessed one eggcell, two synergids, two polar nuclei and several number of antipodals. There was no remarkable deviation from the normal embryo-sac apparatus of the normal *durum* plants. Eggcell at 24 hours after pollination were in the mitotic prophase in both male-sterile and normal plants. Proembryo at 2, 3, 6 and 10 days after pollination did not show any conspicuous difference between both strains [cf. 62, 158].

The increase in length of the embryo during development was measured. The distance between the lower end of suspensor and the upper end of the pro-embryo (or of the scutellum) was measured. During the first 6 days the length showed no noteworthy difference between male-sterile and normal *durum* plants. In the 10 days after pollination, the embryo of male-sterile plants had become longer than that of normal plants in 1956 (Table 9). However, in 1957 both

TABLE 9  
Changes in length of embryo during development in normal and MS *durum* plants

Days after pollination	$M \pm \sigma$ ( $\mu$ )		t	p
	Normal	Male-sterile		
1	40.6 $\pm$ 4.99	35.7 $\pm$ 4.64	1.45	0.2-0.1
3	79.6 $\pm$ 7.22	74.8 $\pm$ 9.14	1.04	0.4-0.3
6	169.7 $\pm$ 18.33	155.2 $\pm$ 90.94	1.17	0.3-0.2
10	534.9 $\pm$ 11.34	823.6 $\pm$ 15.08	46.11	<0.001
Mature embryo	2582.2 $\pm$ 28.42	2404.8 $\pm$ 10.67	17.58	<0.001
Seedling organ	1955.3 $\pm$ 28.42	1806.1 $\pm$ 11.83	4.77	<0.001

groups were pollinated on the same morning. In this case no difference was found between 10 day old embryo of male sterile and normal *durum* plants. In the pollination in *dicoccum* (Kh), which done during a period of lower temperature, smaller embryos were found than those obtained during the later season. From these results it is concluded that the speed of embryo development seems to be greatly dependent on environmental condition.

In mature kernels, however, the length of embryo is larger in normal *durum* plants than in the male-sterile ones, and the length of seedling organe is also greater in normal than in male-sterile plants. Also, there are significant differences between the length of male-sterile and normal plants.

## 2. Reciprocally different cross-compatibility and its embryological aspects

### a. Reciprocal crosses between *Ae. ovata* and Emmer wheats, *durum* (R), *dicoccoides* (K) and *dicoccum* (Kh)

A significantly differently degree of seed-setting was found in different (reciprocal) combinations. For instance, when *ovata* plants were crossed with the pollen of Emmer wheats, a crossability (indicated by seed-setting) as high as 80% was obtained. In the reciprocal pollination, Emmer ♀  $\times$  *ovata* ♂, it was only



5.8-7.1%. No detectable harmful effect was attributable to the presence of gametophytic genes, which might control the function of gametes. At first sight, the cytoplasm of Emmer wheats might be considered as the causal factor for such low crossability. However, even if Emmer wheats with *ovata* cytoplasm were used as female parents, the crossability was very low. Therefore, the low crossability is not attributed to the constant cytoplasm [40].

#### b. *Embryological aspects of reciprocally different crosses*

In order to obtain more detailed information on this point, observations on pollen-tube penetration into foreign stigmata was made, as determined by staining with cotton blue. No noteworthy difference of pollen tube behavior was observed between the reciprocal combinations. Histological observations, however, 2 days after pollination, revealed a remarkable difference in embryo formation; namely, the proembryo, resulting from the pollination of *ovata* ♀ × *durum* ♂, consisted of 2-4 cells, while the eggcell and the polar nuclei in the reciprocal pollination remained undivided. Even at 10 days after pollination the eggcells in the latter did not start nuclear division [40]. From these data it is not obvious whether a failure of embryo formation after the cross-pollination of *durum* ♀ × *ovata* ♂ occurs after the fusion of the nuclei between the eggcell and the sperm, or whether an arrival of the pollen tube into the embryo sac is unsuccessful. There was no indication of hyperplasia of the nucellus or of the inner integument, which was designated as somatoplastic sterility by Cooper and Brink [14]. A further detailed survey concerning this point will be made in the future.

#### c. *Cross-compatibility in reciprocal backcrosses*

The crossability in the backcross between (*durum* × *ovata*) ♀ and *durum* ♂ was 43.2%, while that between (*ovata* × *durum*) ♀ and *durum* ♂ was 48.9%. These crossabilities are not significantly different from the  $\chi^2$ -test. On the other hand, the crossability in the backcross between (*durum* × *ovata*) ♀ and *ovata* ♂ was somewhat higher than that in the backcross between (*ovata* × *durum*) ♀ and *ovata* ♂ [40]. From these results, it may be safely concluded that 1) the difference of crossability in the previous generations was not maintained in subsequent backcrosses, and 2) even if the cytoplasm of F<sub>1</sub> hybrid was different in origin from the pollen parent, used for the backcross pollination (for example,  $\alpha$  AB ×  $\beta$  BB), the crossability was not always lower than in the case where the same cytoplasm was combined (for example,  $\alpha$  AB ×  $\alpha$  AA).

### 3. *Discussion*

In reciprocal hybridization between different species or genera there is occasionally a great difference in degree of crossability. This is especially frequently observed in crosses between plants with different chromosome numbers [70, 115, 154, 158]. However, a difference in crossability between reciprocal crosses has been found even between plants with the same chromosome number. In that case, the difference may be attributable to the different gene constitution of their endosperm [63]. If the pollen-tube would fail to penetrate into a foreign style, however, such a difference in crossability may be attributed to the cytoplasm of the maternal plant.

Schwemmler [135, 136] reported for *Oenothera* on an influence of cytoplasm, even of pollen cytoplasm, and plastids on the degree of compatibility. Hall [49] observed that wheat, which developed from the embryo transplanted on the rye endosperm, had a higher degree of crossability with rye than ordinary wheat. It is not obvious, however, whether the cytoplasm would maintain this property through successive generations. In the present material, when reciprocally different  $F_1$  hybrids, (*durum* ♀ × *ovata* ♂) and (*ovata* ♀ × *durum* ♂) respectively, were pollinated with *durum* pollen there was no difference in the two crossabilities. It can, therefore, be stated that the "incompatible mechanism" which brings about reciprocally different crossabilities is not transmitted to the next generation.

The reciprocal crosses between Emmer wheat and *Ae. ovata*, when the former was used as female parent, show a crossability, which is much lower than that, in which the latter was used as female. There are two possible explanations for the reciprocal difference in crossability. Firstly, as already postulated by Katayama [63] and others, there might be a qualitative difference between fertilized polar nuclei. For instance, in *ovata* ♀ × *durum* ♂, fertilized polar nuclei (endosperm) consist of  $C^u C^u M^o M^o AB$  genomes, while in *durum* ♀ × *ovata* ♂, the nuclei possess  $AABBC^u M^o$ . Secondly, *durum* cytoplasm may disturb the compatibility. If, for instance, *ovata* pollen tubes would not penetrate into the *durum* style, the first assumption is eliminated. However, the pollination in this case very rarely gave fertilized eggs. Therefore, it may be that both possibilities apply, based on 1) the difference of endosperm constituents, 2) the incompatibility between genome constitution of pollen grain and the special status of the female organ which is developed under the control of Emmer genome. It seems to me that the latter (2) is principal causative of the low cross-compatibility.

## VII. SUMMARY

(1) By the nucleus-substitution and -restoration experiments between *Ae. ovata* and *T. durum*, the following points were elucidated: (a) When *durum* genome were transferred into *ovata* cytoplasm, the resulting  $14_{II}$ -plants became completely male-sterile, without exception. (b) The male-sterile factor did not occur in the course of successive backcrossing, or as a result of the amphidiploid condition. (c) When *ovata* genome was transferred into *durum* cytoplasm, the offspring became fertile on both male and female side. (d) When *ovata* genome was replaced again in the cytoplasm of *durum* plants, all resulting  $14_{II}$ -plants became normal, showing high pollen fertility.

(2) It was found that *T. dicoccoides* (*K*) possesses some dominant nuclear genes affecting pollen restoration in MS Emmer wheats with *ovata* cytoplasm.

(3) When MS *durum* plants were crossed with "Einkorn" wheat, the resulting triploid hybrids showed complete sterility on both male and female side.

(4) Pentaploid hybrids from the cross of MS *durum* and Dinkel wheats did not produce good pollen. Further offspring with 21 bivalents, obtained from successive backcrosses, gave typical male-sterile Dinkel wheats.

(5) Substitution of *variabilis* genome into *ovata* cytoplasm showed that *ovata* cytoplasm did not bring about male-sterility.

(6) The interrelationships between cytoplasmic male-sterile factor and fertility-restoring factors, and between genome and cytoplasm concerning male sterility was discussed.

(7) Cyto-histological investigations revealed that the microspore development of MS *durum* plants proceeded normally approximately until the stage of germ-pore formation, and that tapetum abnormalities did not occur. However, malformed anthers, consisting of 3 loculi, were occasionally observed.

(8) Paperchromatographic analysis of free amino acids and sugars in the anthers of MS *durum* plants revealed that the disappearance of proline and the remarkable accumulation of asparagine in the anthers during the course of pollen degeneration, and that a deficiency of sucrose was found in male-sterile anther.

(9) Various abnormalities, besides male-sterility, were frequently found in the plants with foreign cytoplasm. Particularly, the following were noticed: (a) retardation of vegetative growth, (b) reduction in plant height, (c) chlorophyll variegation, (d) abnormal stomata. They did not segregate in subsequent progeny.

(10) The reciprocal difference of cross-compatibility between *ovata* and Emmer wheat was not due to the cytoplasm, but to the incompatibility between the genome constitution of the pollen grains and the special status of the female sex organ.

(11) Morphological observations of developing embryo of male-sterile Emmer wheats showed no evidence of the occurrence of apomixis, and not noteworthy difference between normal *durum* and MS *durum* plants.

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## VARIABILITY OF MORPHOLOGICAL STRUCTURE AND MODE OF REPRODUCTION IN *ENTEROMORPHA LINZA*

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### INTRODUCTION

The algae in the intertidal zone are exposed to many environmental changes, and many variations in their morphological structure and mode of reproduction are recognized. The exact analysis of the environmental factors by which these variations are induced has not been undertaken previously in studies of taxonomy and life-histories of these algae.

*Enteromorpha* is the commonest green alga occurring in the lower tidal zone. The thallus of *Enteromorpha* is essentially tubular with a one-layered wall. While in *Ulva*, this one-layered tube becomes flattened and compressed, and makes a leaf consisting of the double cell-layers which have become fused. *E. linza* is intermediate between these two genera. In this species, the thallus consists of two cell-layers fused with each other excepting along the edge of the blade and the nearest part of the root.

*Enteromorpha linza* has been reported to be reproduced only by quadri-flagellate zoospores. Bliding [2, 3, 4] reported that *E. linza* in Sweden produced only quadri-flagellate swimmers, which germinated directly into a thallus producing again quadri-flagellate zoospores. In addition to the above, Yamada and Saito [14] reported that *E. linza* of Muroran (Hokkaido, Japan) liberated either quadri-flagellate zoospores or bi-flagellate swimmers. They assumed that the bi-flagellate swimmers might be an abnormal form of quadri-flagellate zoospores. Moreover, Moewus [9] reported that gametes were collected in the natural field and also that gametes were obtained from thalli which had been cultured for several generations in his laboratory. Finally, Arasaki [1] observed at Mikawa-Bay, Aichi Prefecture in Japan, that *E. linza* liberated quadri-flagellate zoospores in both of December and January in general and gametes in March and May. It was proved that *E. linza* had two types of reproduction. These observations suggested to us that the method of reproduction of *E. linza* was influenced by environmental conditions depending on localities and seasons concerned.

In order to prove this suggestion the populations of *E. linza* at various places near Tokyo were examined with reference to their morphological structure and mode of reproduction throughout the course of a year.

### MATERIALS AND METHODS

Seven places near Tokyo where *E. linza* occurred were selected for this purpose of study. They were Tokyo-Bay (Shinonome), Miura peninsula (Koajiro,

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Aburatsubo), Jôgashima, Enoshima and Chôshi. These areas selected were delimited by markers. Maturing plants were taken every fifteen days; however, because of variation in environmental factors, they matured irregularly once in a while as they were variable according to the environmental factors, and we could not always get materials available for the study. Swimmers were obtained from thalli that were partially desiccated at first and then returned to filtered sea water. The released swimmers began to swim like a green colored cloud and gathered on the light side or sometimes on the dark side of the Petri dish in which they were released. Samples of them were picked up with a glass pipette and observed under the microscope. Iodine-potassium-iodide was used for staining the flagella of the swimmers. For the purpose to obtain the swimmers, thirty thalli (or less if this required number it was impossible to obtain) were used in each test.

### OBSERVATIONS

The observations made are reported by locality as follows and Fig. 1 presents a summary of them.

#### *Tokyo-Bay (Shinonome)*

February and March. The number of flagella of a single swimmer was indefinite, but varied from zero to four. Bi-flagellate swimmers were generally smaller and more active than quadri-flagellate ones and they showed higher degree of photopositivity. The variation in the number of flagella observed on a single swimmer seemed to be correlated with the type of thallus from which they were liberated. Great variation in the number of flagella was observed in old and broad thalli and large swimmers were often additionally liberated. On the contrary, young and slender thalli regularly liberated bi-flagellate and smaller swimmers.

April. Old and broad thalli disappeared from the marked area late in April and only slender thalli were left, so that only bi-flagellate swimmers were found at that time.

July. After most of the thalli had disappeared from the zone, the bi-flagellate swimmers disappeared. Therefore, only quadri-flagellate swimmers were found in the area. These were not numerous.

November. The thalli collected in autumn were slender and long in their shape, but released quadri-flagellate swimmers instead of the bi-flagellate ones observed in spring.

(September. Disappearing from the marked zone, some were found on one of the piles near by and on shells 100 meters away. These, however, couldn't be identified with *E. linza* because of their peculiar structure. They also liberated quadri-flagellate zoospores that resembled those of *E. linza*.)

#### *Koajiro (rocky shore)*

February. We observed that many abnormal swimmers were liberated late in February. Similar swimmers were liberated at the same time of the last year



at Tokyo-Bay, which suggests that some common seasonal factors might lead to such a phenomenon.

May. Two types of thalli were recognized in this area, slender or string-like form and broad or belt-like form. No correlation was recognized between the forms of thalli and the kind of swarmers.

November. Gamete-like, bi-flagellate small swarmers were released from one thallus collected at this time, and on the next day, the same-sized swarmers were obtained from two other thalli. Conjugation did not occur among these swarmers from the same place but they did conjugate with those collected 300 meters away.

#### *Enoshima*

Quadri-flagellate swarmers were obtained at this locality in the most part of the seasons in a year being contrary to the two places noted above.

February. Many abnormal swarmers were liberated adding to quadri-flagellate swarmers.

October. Bi-flagellate swarmers which were not gametes, were also observed.

(August. Small thalli that liberated gametes were obtained at this time. However, these couldn't be identified with *E. linza* because of their peculiar structure.)

#### *Jôgashima*

February. Thalli collected here seemed to be the same species. However, they were found to be separated into two different varieties, from the careful study on the structure of the thallus.

August. Though the thalli had disappeared at all other places in August, many large thalli appeared here. It seemed that they developed from swarmers which were newly fixed.

November. Half of the thalli were observed to be gametophytes, and they were similar to sporophytes in the shape of them.

#### *Chôshi*

March. The slender thalli in the upper tidal zone were almost all sporophytes, and broad thalli at the lower tidal zone were almost all gametophytes. Sporophytes and gametophytes were found to occur in different zones.

May. No ecological difference in location between gametophytes and sporophytes was recognized at this time, being contrary to the observation just referred above.

July. The gametes which had been in abundance till June were observed to have diminished in number at this time, and they didn't conjugate with each other.

August. While the number of the flagella of swarmers was variable, most of swarmers were bi-flagellate. They didn't conjugate, but swam photopositively.

October. Many thalli began to appear again in the same field. They liberated bi-flagellate swarmers in great numbers as in the withered season in August.

December. No mature thalli could not be found in the field. Nevertheless, it

seemed that the kind of swimmers was similar to the one as shown in Fig. 1, because as a result of culture in the laboratory, three kinds of swimmers were obtained: bi-flagellate, quadri-flagellate swimmers and also gametes.

*Koajiro (sandy shore)*

January. Most of the gametes were the same sex. One hundred thalli were observed through 4 days and only two sporophytes were found.

May. All thalli were gametophytes which consisted of male and female what were in almost same ratio.

June. Sporophytes appeared early in June, and morphological differences were not found between these sporophytes and the gametophytes.

November. The same species appeared also about 100 meters distant from the original locality under the same ecological conditions.

*Aburatsubo*

*E. linza* occurs spreading over a rather wide area mixed with other *linza*-like species, so it is difficult to identify true *linza* especially in withered seasons. The typical thalli of *E. linza* collected in this area were always observed that they liberated quadri-flagellate zoospores.

Through several phenomena observed as the above, the followings are of general interest:

1. *Bi-flagellate asexual swimmers*

Two different types of asexual reproduction were observed in *E. linza*: One of them was by asexual bi-flagellate swimmers (Tokyo-Bay and Koajiro), and the other was by quadri-flagellate swimmers (Enoshima and Jôgashima). However, different kinds of swimmers occurred usually at the beginning or end of the season when thalli were to be found: bi-flagellate swimmers were found at Enoshima in October, and at Chôshi in August and October, and quadri-flagellate swimmers were found at Tokyo-Bay in summer and autumn.

The number of flagella is variable—zero to four, or five by chance—under heavy rain in the field, or under postponed or forced liberation of swimmers in the laboratory. These might be caused through irregular division, and such abnormal cases were observed to occur rather often in the thalli which were expected to liberate quadri-flagellate swimmers.

Bi-flagellate swimmers had some similarity to the gametes in their higher degree of photopositivity, quick movement and smaller size. However, they never conjugate with each other nor with true gametes.

2. *Differences in the liberation time of zoospores and gametes*

a) Quadri-flagellate zoospores were produced in Petri dishes from all the thalli collected on the 10th of December at Misaki near Aburatsubo, while on the next day, many bi-flagellate smaller swimmers were found in the same dishes containing these thalli. These bi-flagellate swimmers were gametes, for they conjugated with each other.

The re-examination of freshly collected materials was made on the 19th of

the same month at the same place. All the fronds examined liberated only quadri-flagellate swimmers on the first day of examination, however, on the next day some gametes were found in the Petri dish even though no gametophytes could be identified at this time in the field.

b) Gamete formation took place at one of the two places in Jôgashima facing each other about 200 meters away from the area of the study. Here only a few mature gametophytes were found at the latter on the day following.

Two kinds of thalli which resembled each other shaping similarly were collected from two different places. One of them liberated both bi-flagellate zoospores and gametes, in spite of the other one liberated only gametes. This difference was observed until the thalli at these places had completed their first maturation. At the next tide, half a month later, the latter type of thallus also liberated zoospores.

### 3. The season in which gametes appeared

Gametes were not found throughout the year, as observed by Arasaki at Mikawa-Bay. For example, at Chôshi and Jôgashima gametes were found only in winter, and at Koajiro and Enoshima a few thalli liberating gametes were obtained only at definite season.

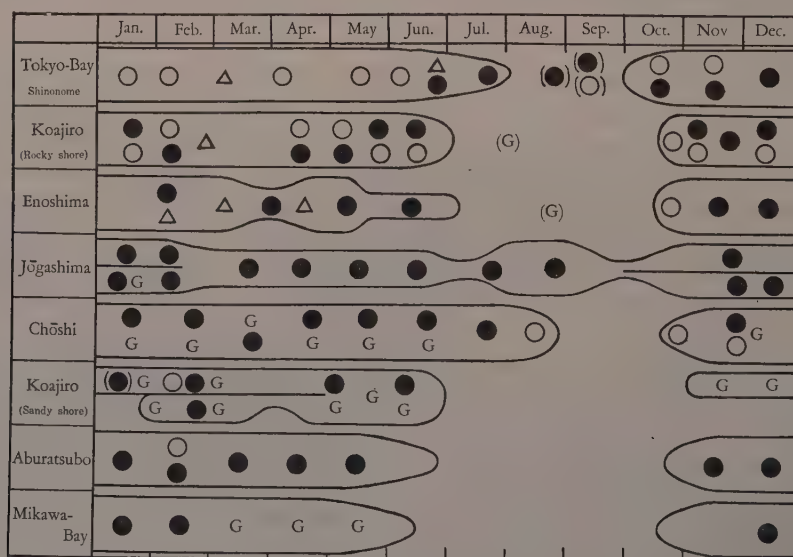


Fig. 1. Seasonal variations of growth of thalli and appearance of spores of *E. linza* at various places.

- quadri-flagellate zoospore
- bi-flagellate asexual swarmer
- △ abnormal swarmer
- G gamete

### 4. Morphological variation

a) At Chôshi in March, the sporophytes were slender and grew in the upper



part of the intertidal zone, and the gametophytes were broad and grew in the lower part.

At Jogashima, no gametes were found in March, granting that they were found in November and January. These gametophytes were slender and grew

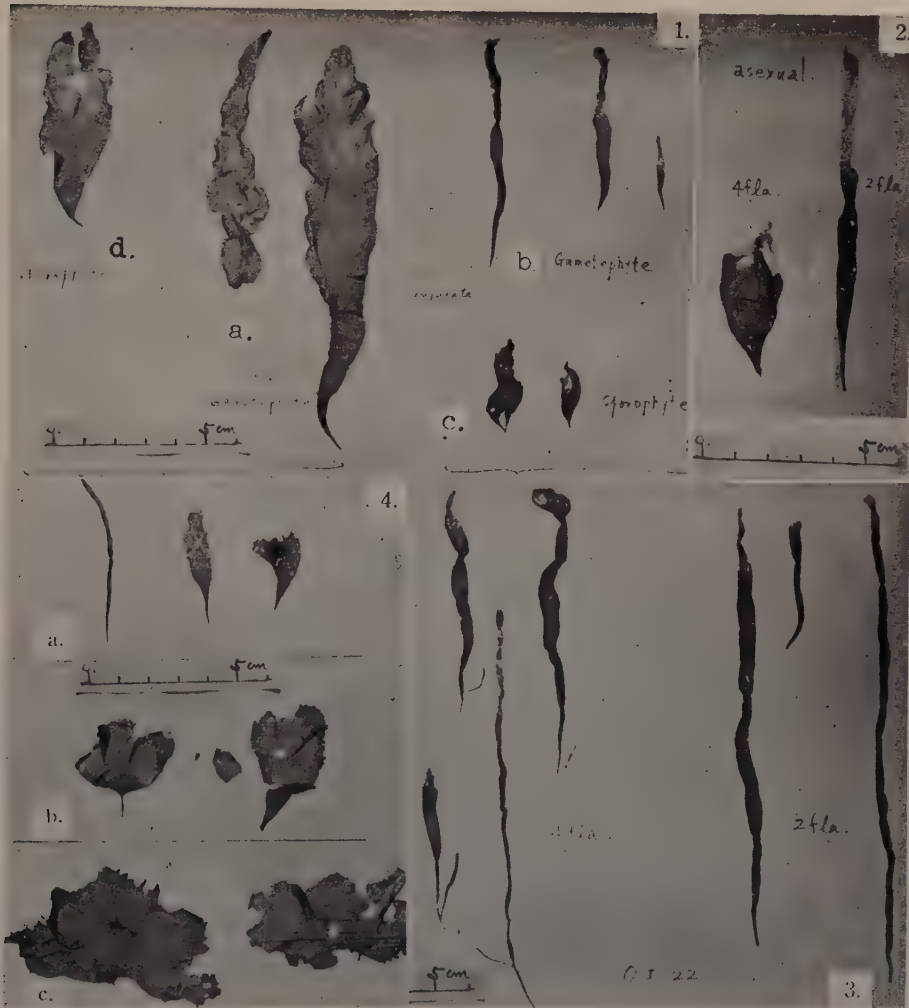


Fig. 2. 1. Gametophytes (a, b) and sporophytes (c, d) collected from two different places 200 meters far at Jogashima. Gametes from a conjugated with those from b. 2. Fronds collected at Koajiro: right: liberated bi-flagellate asexual swimmers; left: liberated quadri-flagellate zoospores. 3. Fronds collected at Tokyo-Bay: right three: liberated bi-flagellate swimmers; left four: liberated quadri-flagellate swimmers. 4. Variation of shape relating to the difference of growing-zone at Enoshima. a. Upper tidal zone near high-water-line. b. Middle tidal zone. c. Lower tidal zone near low-water-line.

in the upper part of the intertidal zone, while the sporophytes grew in a reverse manner (Fig. 2-1). Remarkable morphological differences between sporophytes and gametophytes were observed in some cases, but in most cases, such differences were not found.

b) At Tokyo-Bay in spring and at Koajiro in winter, slender thalli liberated bi-flagellate swarmers, and broad ones liberated quadri-flagellate swarmers (Fig. 2-2). However, we found that all the facts as aforementioned were reversed at Tokyo-Bay in early winter and also at Koajiro in spring. Both types of swarmers germinated in the same way and developed into string-like thalli almost 1 mm in width. These fine thalli could liberate bi-flagellate swarmers.

c) Thalli at two different places at about 200 meters distance were of different shapes, and the gametes produced from them could conjugate with each other (Fig. 2-1).

## DISCUSSION

Our observations in general indicate that some environmental factors control the reproduction of gametophytes and sporophytes of *E. linza* as well as the the number of flagella of swarmers and the difference of their forms.

Moewus [9] reported that, in *E. linza*, gametes, haploid zoospores and diploid planogonidia were present. Bliding reported that *E. biflagellata* always produced only bi-flagellate swarmer which developed asexually. There are reports that some ulvaceous algae always reproduce asexually by means of zoospores. However, our observation shows that if elaborate ecological studies are undertaken, the sexual phase of those species might be found in nature field. Kylin [7] reported that the zoospores of *Ulva lactuca* were not found at the west coast of Sweden; however, Föyn [6] stated that they would probably have been found if small and incomplete thalli were sought for because sporophytes matured and liberated spores even when they could not become large enough to be realized. Smith G. [12] found that in *Ulva* of the west coast of the United States that there was a difference of 3-5 days between gamete maturation and zoospore one. Such a tendency was also recognized in our observations.

Kylin and Bliding stated that morphological and ecological factors should be considered in studying the taxonomy of *Enteromorpha*, and also that in the identification of species observations on gamete-conjugation are necessary. In spite of these statements, Föyn [6] reported that the gametes liberated from two thalli, which were identified as belonging to the same species *Ulva lactuca*, conjugated making many zygotes that did not grow further. He therefore described these thalli as belonging to two different species, *Ulva lactuca* and *Ulva thureti*. The failure of zygotes to grow suggests that the ability to conjugate may not always be proof that the conjugants are of the same species.

Finally, we should pay attention to Drew's report (1955). She pointed out the incompleteness of classification of algae when only somatic characters were used. She said that so-called life-histories depend upon both somatic characteristics and nuclear characteristics and tried to classify the life-histories of algae with the

help of studies on nuclei. According to Drew there are three morphological types, the monomorphic type, the dimorphic type, and the trimorphic type. In the nuclear phase there are two types, the diploid and the haploid. Therefore, through the combinations of the morphological and nuclear types, the class Chlorophyta can be divided into the following four groups; the monomorphic diplo-haplonts, the monomorphic diplonts, the dimorphic haplonts, and the dimorphic plants that cannot be identified with certainty but unknown whether diplo-haplonts or not. She proposed that the *Enteromorpha* belongs to the first group, the monomorphic diplo-haplonts, but she excluded the *E. linza* from this group because of the absence of gametes. Standing on our observations we cannot agree with her opinion, and we think that the *E. linza* should be included in the same group as the other species of *Enteromorpha*.

As a whole, we speculated that in such primitive multi-cellular plants as *Enteromorpha*, several morphological types, somatic phases and so forth are non-genetic, and may be changeable as the result of adaptation to environmental factors. Another conclusion is that the taxonomy in *Enteromorpha* should be considered again from the point of view of the influence of environmental factors upon the display of characteristics, or morphogenesis.

### SUMMARY

The phenology of *Enteromorpha linza*, a common alga, occurring luxuriantly from autumn to late spring at several places near Tokyo was studied throughout the year. In these seasons, the mode of reproduction or the form of individual thalli was found to be quite variable from place to place and from season to season.

*E. linza* can be separated into two types from the mode of reproduction. Firstly it is the principal method to make use of sexual reproduction and secondly it is that asexual reproduction is the principal method. In the former type, the ratio of gametophytes to sporophytes was different depending on seasons, and also the same in case of the ratio of males to females. In some areas gametophytes and sporophytes were distinguishable in their shapes or in ecological preferences, but became difficult to distinguish in other areas. In the latter type, the swarmers could usually develop asexually; among them quadri-flagellate swarmers and bi-flagellate swarmers were observed. Also, the form of the thalli which produced bi-flagellate swarmers was different in some cases and in the other case they were all similar. The ratio and the ecological preference of the fronds whether producing quadri-flagellate swarmers or bi-flagellate swarmers were variable.

From the observations and experiments mentioned above we concluded that the morphological structure and mode of reproduction of *Enteromorpha linza* can be altered through the influence of environmental factors. Therefore, we suggest that students should be prudent in dealing with the taxonomy and life-history of *Enteromorpha*.



Acknowledgements are made to the staff of Misaki Marine Biological Station, the staff of the laboratory of Enoshima Aquarium, and Mr. H. Yoshida of Suma Aquarium all of whom supported our investigation. We also thank Prof. John E. Cushing of the University of California, Santa Barbara, and Dr. K. Miyake for their suggestions concerning the preparation of the manuscript.

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## OBSERVATIONS ON THE STRUCTURE AND ECOLOGY OF A XEROPHYTIC *SELAGINELLA* FROM INDIA

### I. *SELAGINELLA BRYOPTERIS* (L.) BAKER

N. P. CHOWDHURY

#### INTRODUCTION

Contributions to our knowledge of Indian *Selaginella* have been rare, in fact, the genus is one of the least known in the Indian Flora. A few valuable contributions are from Bancroft [3], Ghosh [17, 18], Ogura [38], Majumdar [34, 35] and Alston [1] on species mostly of humid habitations, but practically nothing is known about the interesting group of the xerophytic species of *Selaginella* inhabiting comparatively exposed and dry areas of our country. The author, therefore, at the suggestion of the late Professor Birbal Sahni, undertook the study of some xerophytic species of Indian *Selaginella*. The present investigation is a study of the structure and ecological anatomy as well as of some features of special interest of *Selaginella bryopteris* (L.) Baker, a rare xerophytic species of restricted distribution (Chowdhury [9, 36, 39]).

The material of *S. bryopteris* was originally collected in 1929 by Dr. S. K. Pande of the Lucknow University from Karvi in the district of Banda (U. P.) and later by the author (1954) from other localities. The scattered rocky hills of Banda district reach at points a height of 1300 ft. (ca. 396 m) above sea level [28]. The hills are well wooded and abound in massive boulders, gigantic scarps and deep ravines. The average recorded rainfall in the district is 1013 mm. Some of the droughts of the place have been remarkable, namely the one of 1880 with only 452 mm rainfall [12].

The author also found the dried plants of *S. bryopteris* being sold by local herbalists (Dehra Dun 1950, Delhi 1954) under the names of "Sanjeevani", "Hanspadi", "Pasana bhedana", etc. with reputed medicinal property as a nerve and brain tonic attributed to it according to the Ayurvedic system of medicine.

#### MATERIALS AND METHODS

The material was preserved in 70% alcohol and was subsequently transferred to a mixture of 70% alcohol and glycerine. The material being hard and woody n. butyl alcohol was preferred to xylol for clearing, as the former had a softening action on the hard woody tissues [53]. Very often the harder portions had to be softened by keeping them in 20-30% hydrofluoric acid [7, 31] for 8-10 days and finally clearing them in n. butyl alcohol. Meyer's albumen was found inconvenient; the sections being mostly woody and rather thick (12-20  $\mu$ ), were often washed off in the process of staining, etc. Land's method of fixing

paraffin sections by gloy (starch glue) and potassium dichromate solution was tried with advantage [30, 33]. The stain combination safranin-fast green gave all round good results. Other combinations like safranin anilin blue, crystal violet-orange G. and erythrosin-iodine green or erythrosin-crystal violet also gave fairly satisfactory results. Much of the sectioning was also done free hand or by a wood or a hand microtome using stems of *Tinospora cordifolia* Miers.\* as pith for holding the material.

## DESCRIPTION

*Selaginella bryopteris* (L.) Baker in Journ. Botany XXII, (1884), 376; Alston in Journ. Botany LXIX, (1931), 252, and Proc. Nat. Inst. Sci. India. XI, (1945), 221.

*Synonym:* *S. imbricata* (Roxb.) Scott in Journ. Agri. Hort. Soc. N.S.I., (1868), 260; Edgeworth, Cat. Pl. Banda Distr. (1852), 38; *S. tamariscina* (P.B.) Spr.; *Lycopodium bryopteris* Linn. Sp. Pl. (1753), 1103.

*Systematic position:* It belongs to the subgenus 'Heterophyllum', section 'Monostelieae' and to the group *S. lepidophylla* of Hieronymus [15] or subgenus 'Stachygynandrum', series 'Rosulatae' of Baker [2]. Some of the allied species are *S. involvens* (Sw.) Spr.; *S. convoluta* Spr.; *S. pilifera* Spr.; *S. cuspidata* Link.; *S. lepidophylla* Spr.; *S. pringlei* Bak.; *S. imbricata* Spr.; *S. orbigniana* Spr.; *S. longispicata* Underw.; *S. stauntoriana* Spr.

### 1. *External Features* (Figs. 5, 6, 7, 18, 19, 20, 28).

The plants are 5-10 cm high, spreading, densely tufted, simple in the lower two-third but the upper third deltoid and decompound. The upper and ultimate branches are more or less flabellate and markedly dorsiventral. The species resembles closely *S. imbricata* Spr. in habit. The individuals, however, do not form flat dense rosettes like the allied species with inrolling habit, but the stems are more or less erect. The lower part is unbranched and covered with a few scale-like leaves; the upper half of the stem is branched as in the other species of the group. The leaves of the upper branches are dimorphic. The ventral leaves are much imbricated, obscurely serrate, adpressed, ovate, orientated more or less horizontally on both sides of the stem, cuspidate, firm and rigid in texture (Figs. 6, 7, 18). The dorsal leaves are nearly as long, ascending oblique ovate with a large cusp (Figs. 5, 18). Cones short, square in transverse section; sporophylls ovate, cuspidate and keeled [2, 24]. Leaves ligulate (Figs. 5, 6).

The rhizophores could not be traced. The dark brown 'prop-like' structures descending down either from the trailing upper stem or from the basal upright axis without any relation to the branching of the axis are roots having a prominent cover of root hairs throughout (Figs. 20, 28). Sinha [44] remarked that the species has no rhizophores. Gibson [21] observed *S. cuspidata*, *S. lepidophylla*, *S. involvens* and *S. pilifera* to be devoid of rhizophores\*\*; and he considered the

\* I am indebted to Dr. Bahadur Singh, of B.R. College, Agra, for suggesting this.

\*\* Dr. T. M. Harris, of the University of Reading, when discussing the paper with the author at the Palaeobotanical Institute, Lucknow, (Jan., 1950), remarked that rhizophores are reduced in these species. The author is thankful to him for the valuable suggestions offered.



absence of so called rhizophores to be rather characteristic of the types having dense tuft of aerial erect axes arising from short rhizomic bases as in *S. pilifera* and *S. involvens*.

The plants are well fitted for a semi-arid environment. During drought the stems (the upper portion) curl up into a close cluster. The under sides of the ventral leaves which become the outside of the cluster in the curled up condition look silvery glossy and therefore reflect much of the light they receive. Their cells contain numerous oil globules which could be tested with Sudan III or Alkanin ([7] p. 84, [40] p. 58) and which evidently serve to protect against extremes of hot or cold.

## 2. Anatomy

*Stem* (Figs. 1, 2, 3, 8, 9, 10, 22, 23, 29, Text-fig. 2). The stem, as already stated, exhibits a definite dorsiventrality in the upper branches and is monostelic. The lower erect and old portion of the stem is covered with scaly leaves all around as was observed by Uphof [47] in *S. imbricata*, and in transverse section shows a layer of elongated epidermal cells with thick outer walls as in *S. involvens*, *S. lepidophylla* [19]. The epidermis is followed by 2-3 layers of large thin-walled cells composing the outer cortex. A zone of 7-8 cells layers deep, thick-walled sclerenchymatous middle cortex is very prominent; this is succeeded by 4-6 layered thin-walled inner cortex of large cells (Fig. 1). The stereome is developed chiefly in the erect shoots. A narrow lacuna with loosely packed 2-celled trabeculae (endodermis) holds the stele consisting of 2-4 layers of pericycle, protophloem elements, a layer of sieve tubes, a few layers of phloem parenchyma and the central semicircular mass of tracheids with 3-4 marginal protoxylem groups (Figs. 1, 2). Sinha [44] had failed to find a trabecular endodermis in old stems. In the creeping axes of some allied species, namely *S. Lyallii*, *S. inaequalifolia*, practically no lacuna is to be seen [19]. The dorsiventrally flattened sieve tubes, as also observed by Gibson [19] in *S. lepidophylla*, *S. pilifera*, *S. convoluta*, form a complete ring but imperfectly developed opposite the protoxylem groups.

Higher up, the outer cortex widens, the sclerenchymatous middle zone narrows down and gradually, in the dorsiventral ultimate branch becomes localised to a layer of sclerenchymatous cells on the lower (ventral) surface of the stem forming a flange (Figs. 3, 22, 23, 29). With the increase in the size of outer cortex its cells become large and thick-walled, especially those of the dorsal side and the inner cortex, as well as the bulk of the stelar tracheids are reduced. The lacuna becomes more prominent with loosely packed 2 or more celled trabeculae\* consisting of a short endodermal cell articulating inwardly with a pericycle cell and outwardly with one or more inner cortical cells. Vladescu [48] had observed that each endodermal cell articulated with two cells of the pericycle on the one hand and two cells of the cortex on the other. De Bary [11] and Russow [41] considered a true endodermis to be absent, but Leclerc du Sablon [32] thought that the cuticularized cells which arise from the limiting layers of the

\* The trabecula is sometimes merely an endodermal cell; at other times one, two, or more parenchymatous cells are connected with the endodermal cell to form the tubercula (Gibson, [19], p. 147).

vascular cord form a 'genuine endodermis'. Vladescu [48], however, showed in *Selaginella* a common origin for the so-called pericycle, endodermis and a certain number of the inner layers of the cortex; these according to him are only modified layers of the general cortex. Gibson [19] distinguished the endodermal cells as cuticularized cells arising from the chlorophyllaceous pericycle layer surrounding the phloem and used the term trabecula in a general sense for the uni or multicellular strands which anchor the vascular cord to the cortex. The endodermal cell, as described by him, is commonly a tubular cell possessing protoplasm and a nucleus, but no chlorophyll and provided with a cuticularized Casparian band. The protoploem elements which occur immediately within the pericycle, are a few delicate, half or wholly occluded bright-walled cells as in *S. Martensii*; *S. Wallichii* (Gibson [19], Pl. IX, Fig. 2, Pl. XI, Fig. 59). The xylem is composed entirely of tracheids; no vessels could be seen. In a transverse section two foliar traces, a dorsal and a ventral, are seen (Fig. 29), as in *S. Martensii* described by Gibson [19].

The upper branches show marked dorsiventrality with dorsal outer cortex composed of large, well stretched, turgid, thick-walled 7-8 layers of round cells, which I have termed 'motor cells'. They seem to be hygroscopic capable of distending and contracting in size with the absorption or loss of water respectively (Figs. 3, 22, 23, 29). These cells have transverse pittings on their dorsal and ventral walls which are well seen in a median longitudinal section (Text-fig. 2). Campbell [6] also spoke of deeply pitted walls of the cortical cell in the allied species, *S. lepidophylla*. Another peculiarity observed in these 'motor cells' is the presence of tubular spaces in the middle lamella of the walls at the angle of the cells (Fig. 8); this structure strongly recalls the condition found in the thick-walled cortex of *S. Lyallii* described by Gibson [19]. The pitting and the tubular spaces obviously aid in the function of expansion and contraction of the 'motor cells' causing the unfurling and inrolling of the branches. The inner cortex is composed of thin-walled parenchymatous cells (Fig. 9) and is a much reduced 1-2 layered narrow zone dorsally but quite a broad and wide 5-6 layered zone ventrally, where the outer cortex is narrowed to a thin strip of sclerenchyma which lies immediately beneath the epidermis (Figs. 10, 23, 29) and serves as a mechanical strengthening flange or shield to withstand the pull exerted on the ventral surface, when the branches incurd dorsally by the contraction of the 'motor cells' during drought conditions. The significance of the structural difference of the dorsal and ventral tissues of the upper branches is thus well seen which is responsible for the characteristic inrolling movement of the branches, which appears to be purely hygroscopic and mechanical. This phenomenon is further discussed later. The stele in this region of the stem, although composed of the same tissues as described above for the lower or basal region, is markedly dorsiventral with protoxylem elements situated on the two lateral margins only (Figs. 3, 23, 29).

The anatomical structure of the stem thus varies considerably in the different parts and no one region can be regarded as showing a typical structure. The extreme plasticity of anatomical structures in the members of Lycopodinae

is well known through the researches of Holloway [27], Wardlaw [49, 50], Bower [4], Chowdhury [8], etc. in the species of *Lycopodium*. Spring [45], Gibson [19], Bower [4], p. 33) drew attentions to the marked differences in anatomical structures seen in many cases between the basal (procumbent) axes and erect upper portions of the stem in *Selaginella* species, a distinction curiously enough ignored by many later anatomists.

*Leaf* (Figs. 5, 6, 7, 11, 12, 13, 14, 19, 27). The structure of leaf in *Selaginella* has been described at length by Hieronymus [15], Gibson [20], Russow [41], Satake [43] and others (Dangeard, Erikson, McNab) as cited by Gibson, and hardly need any more comment here except for the special features exclusive to this species.

The dorsal leaves are ovoid oblique with pointed apices (Fig. 5) and 2-3 layered spiny sclerotic margins. A transverse section through the oblique cuspidate leaf shows the epidermis of both the ligular and the aligular surfaces to be composed of wavy-margined elongated cells. The upper epidermal cells are thicker-walled and with narrower lumina (Fig. 11, 12, 27). There is a well developed double layered palisade region on the upper surface beneath the hypodermis and a reticulate mesophyll lower down. The occurrence of a double layer of palisade is very peculiar and so far is recorded only in *S. Lyallii* [6] and *S. concinna* [20], where also it is not so prominent. In this respect it differs also from the leaf of *S. lepidophylla* where the mesophyll consists of reticulate parenchyma only [15]. *S. involvens* and *S. pilifera* which closely resemble *S. lepidophylla* have the cells of the reticulate mesophyll "more closely packed forming a pseudo-palisade layer" [20] and are far from being so specialized as in *S. bryopteris*. The structure is also different from those of *S. Martensii* type, e.g., *S. Martensii*, *S. cuspidata* and *S. convoluta*, where the mesophyll consists of reticulate parenchyma alone, and the epidermal cells are conical and peg shaped in section with broader end facing outward, the epidermis of the ligular and aligular surfaces is dissimilar [20]. In *S. convoluta*, however, the reticulate mesophyll is closely packed on the upper surface forming a pseudo-palisade layer [20].

The ventral leaves of *S. bryopteris* are broadly ovate, cuspidate (Fig. 6) and very unequal sided comparable to those of *S. pilifera*, *S. Martensii*, *S. cuspidata* and *S. concinna* described by Gibson [20]. Of the two stiff hair fringed halves of the leaf one lying more or less beneath the stem is very much expanded and furcate and is really a broad sheet of 4-6 layered sclerotic fibres gradually thinning on the margin to a single cell thickness (cf. *S. concinna*, *S. cuspidata*) (Figs. 6, 14). The other half of the ventral leaf is similar in size and lies somewhat on the side of the stem; this half is much thicker in texture and has a dark brown pigment developed on its surface as in *S. pilifera* described by Uphof [47]. In this leaf there is a well developed palisade layer beneath the upper epidermis and a reticulate mesophyll towards the lower surface (Fig. 13). The margin consists of chlorophyll-free elongated sclerotic cells from which unicellular hairs arise. The chlorophyll-free margins of the leaves obviously reflect light and shield the parts they cover against strong sun and heat.



As described by Rusow [41], the vascular bundle terminates before the apex of the leaf is reached. It is concentric being composed of 4–5 delicate tracheids which according to Dangeard [10] are protoxylem elements surrounded by a scanty phloem and parenchyma without any intercellular space. No endodermis is developed. The cells of the aligular surface of the dorsal leaves correspond to those of the ligular surface of the ventral leaves and vice versa as in *S. sanguinolenta* [22] (Fig. 19). Stomata are seen to occur on both surfaces of both the dorsal and ventral leaves as in *S. cuspidata*, *S. pilifera*, *S. Kraussiana* and *S. lepidophylla* reported by Gibson [20]. The stomata are however concentrated as a rule over the midrib on the aligular surface of the leaf as also found in *S. Wightii* by the author. Dangeard [10] had described the stomata to occur on both sides of the leaf in Rosulatae, e.g., *S. convoluta*, *S. cuspidata*.

The leaf structures in *S. bryopteris* more or less conform to Dangeard's classification [10], of two distinct types e.g., (I) upper and lower epidermis similar but mesophyll homo- (e.g., *S. spinosa*) or heterogeneous (e.g., subdivided into upper palisade and lower spongy layers as in *S. Kraussiana*), (II) upper and lower epidermis dissimilar but mesophyll homogeneous (reticulate) throughout. Erikson [16] on the other hand regarded the presence or absence of a palisade layer as more important and classified the groups accordingly.

*Sporangial region* (Figs. 24, 25). The microsporangium wall is two layered. The outer layer is composed of radially elongated rectangular cells with inner and radial walls thickened. The inner layer is composed of tangentially elongated thin-walled cells with granular contents. The third layer, the persisting tapetum of thin-walled, narrow, papillate cells with dense granular contents abut on the spore mass and disorganized spore mother cells. The microspores are numerous and possess thick brown warty exine (Fig. 24) and measure 38–57  $\mu$  across. In a megasporangium which occurs at the base of the spike only three well developed megaspores (cf. *S. cuspidata* described by Mitchell [37]) are seen with very heavy and unevenly thickened sculptured walls which get broken on sectioning (Fig. 24) and measure 190–247  $\mu$  in diameter. The micro- and megasporangia measured 0.29 mm and 0.54 mm respectively in diameter.

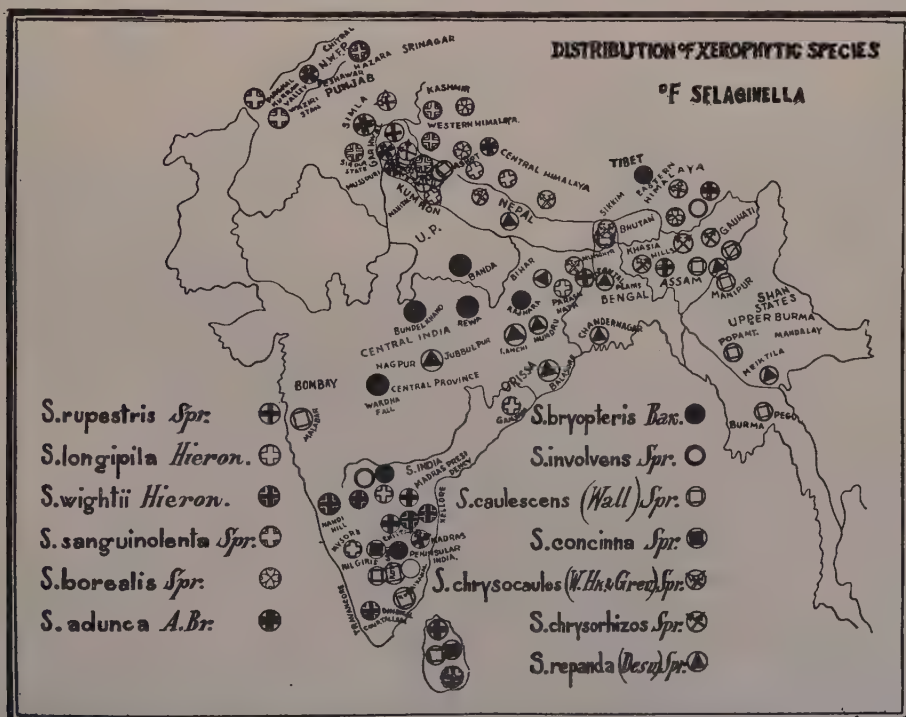
The cone stele (Fig. 25) which is composed of a pericycle layer of big oval chlorophyll containing cells followed by an imperfect (especially opposite protoxylem) layer of dorsiventrally flattened thin-walled sieve tubes, two layers of small cells of phloem parenchyma rich in granular contents and a central plate of tracheids with two marginal protoxylem groups, is suspended in a wide lacuna by well developed endodermal trabeculae (Figs. 24, 25). The trabeculae are two celled, the inner short broad tangentially thick-walled endodermal cell, articulating with the outer long tubular cell which is distally continued to the inner cortex (Fig. 25). Some trabeculae are three celled made up of one endodermal and two cortical cells. The cortex region of the spike is about three layers deep with the innermost layer of inner cortex demarcating the lacuna.

*Apical cell of Selaginella stem* (Figs. 4, 26). Bruchman\* published a short account of his researches on *S. spinulosa* A. Br. in which he stated that the

\* Cited by Gibson [19], p. 141.

apical region of the stem and branches is occupied by a group of cells and not a single cell as Treub [46] had demonstrated for *S. Martensii*. In those species of *Selaginella* which bear leaves in four rows, the apical cell of the stem according to Pfeffer (as cited by Sachs [42]), two edged. The two edges of the apical cell are more or less vertically directed. According to Russow ([41], p. 176) in *S. arborescens*, *S. pervillei* and *S. Wallichii* there is not a single apical cell, but an apical group of 'common initial cells'. Strasburger (as cited by de Bary, [11]) found in *S. Wallichii* two cells in place of one apical cell, which form segments in conjunction with one another. Each of them has the form of a wedge and bounded by five planes. De Bary [11] reported a two-sided cell in the apex of the stem of *S. Martensii* and *S. Kraussiana*. In the seedling of *S. Martensii* apical cell of the main shoot and of the two branches of the first bifurcation has the form of a four-sided pyramid which soon passes back to the two-edged form (de Bary, [11], p. 16). A longitudinal section through the apex of the cone of *S. bryopteris* revealed the presence of a wedge shaped, two sided apical cell (Figs. 4, 26) forming segments from its two sides as described by de Bary [11] in *S. Martensii*.

**Root (Fig. 21).** The root shows in transverse section superficially a piliferous layer of cubical cells often crowned by an unicellular root hair; 1-2 layers of thin-walled, large ovoid cells of outer cortex, succeeded by 5 or 6 layers of small celled sclerenchymatous mid cortex and a thin-walled, larger celled paren-



Text-fig. 1. Distribution map of xerophytic species of *Selaginella*.

chymatous inner cortex, a well marked endodermis, pericycle and a normal *monarch*\* vascular bundle. The phloem surrounds the xylem completely, but apparently sieve-tubes are not developed opposite the protoxylem. The root cortex outside the zone of sclerenchyma is lacunar [44]. The structure on the whole closely resembles with that of *S. cuspidata*, *S. lepidophylla* and *S. involvens* described by Gibson [21].

### 3. Distribution of xerophytic species of *Selaginella* in India, Burma, Ceylon, Pakistan, Nepal and Tibet

The following is an enumeration of the xerophytic species of *Selaginella* as are known to occur in the region mentioned above. The information with regard to their distribution has been obtained from herbarium records, personal collections and relevant literature\*\* (Text-fig. 1).

a) *S. rupestris*, Spr. *Himalayas*<sup>1</sup>: Kali valley (U. P.), 7-8000 ft. (coll. J. F. Duthie)<sup>2</sup>; Garhwal (coll. Griffith)<sup>4</sup>. *Madras Prov.*<sup>4</sup>: (coll. Wallich, Schutter, Griffith)<sup>5</sup>; Pingee (coll. Wight)<sup>4</sup>. *Mts. of Ceylon*<sup>5</sup>.

b) *S. longipila*, Hieron. (mountains of India at higher altitudes). *Himalayas*<sup>3</sup>: Kali valley, 7-8000 ft. (coll. J. F. Duthie, 3727)<sup>9</sup>; Kumaon (coll. Davidson)<sup>9</sup>. *Bhutan* (coll. Griffith)<sup>10</sup>. *Bihar*: Parasnath<sup>2</sup>; Ranchi<sup>10</sup>. *Orissa*: Sambalpur (Kathpahar, coll. H. F. Mooney, 3318)<sup>9</sup>. *Madras Prov.*<sup>2</sup>: Ganjam<sup>2,9</sup> (Mahendragiri<sup>9</sup>, coll. J. S. Gamble<sup>2</sup>, 14138); Nilgiris<sup>2</sup> (coll. Gamble<sup>2</sup>, 7800 ft.); Ooty (Tigerden, coll. Vicary)<sup>2</sup>.

c) *S. Wightii*, (Hieron.) Alston. (confined to S. India and Ceylon). *Ostindien*<sup>3</sup>. *S. India*: Madras<sup>2</sup> (Nellora)<sup>2</sup>; Anantpur<sup>2</sup>—2000 ft. 21154<sup>2</sup> (coll. J. S. Gamble, 1889); Courtallam<sup>2</sup> (coll. M. Rama Rao, 1979)<sup>2</sup>; Veligonda hills (coll. M. S. Ramaswami, 1385)<sup>2</sup>; Voyal pad<sup>2</sup> (Chittoor Dt.)<sup>2</sup>, edges of rocks, 2700 ft. (coll. C. E. C. Fischer, 4349)<sup>2</sup>; Nandi hills (Mysore), 5000 ft. (coll. M. O. P. Iyengar, R. V. Seshaya)<sup>11</sup>; Mudumpadu (Cuddapah)<sup>9,10</sup>; Coromandel (coll. Koenig)<sup>10</sup>; Coorg (?)<sup>9</sup>. *Ceylon*<sup>2</sup>: Dambulla rocks<sup>10</sup>.

d) *S. sanguinolenta*, Spr. (in India mostly). *Western Himalayas*: Kurram valley<sup>6</sup> (W. Pakistan, coll. Dr. J. E. J. Aitchison)<sup>2</sup>; Pirghal (Waziristan, W. Pak. coll. Har Sukh, 15622)<sup>2,9</sup>; Kashmir (Baltal, Pahlgam, coll. Stewart)<sup>10</sup>.

*S. sanguinolenta* var. *Jacquemontii*. *Himalayas*<sup>2</sup>: Kashmir<sup>2</sup> (No. 1231. Chitral relief expedition, coll. Lieut. Harris)<sup>2</sup>; Damdar valley<sup>2</sup>, 10-11000 ft. (coll. Duthie)<sup>2</sup>. *W. Pakistan*: Hazara Dt.<sup>2</sup>, Dogahbela<sup>2</sup>, Shimkyari<sup>2</sup> (coll. Inayat).

*S. sanguinolenta* forma *Indica* (Milde) Alston. (= ? *S. Jacquemontii*). *Himalayas*: Teri Garhwal (coll. J. F. Duthie, 189, 509)<sup>9</sup>; Damdar valley<sup>9</sup>; Kagon valley (Hazara, coll. Inayat, 20807)<sup>9</sup>; Kashmir (Surind valley, coll. J. F. Duthie, 11466)<sup>9</sup>.

e) *S. borealis*, Spr. (*S. Jacquemontii*, Spr.). (Himalayas, China, Siberia). (Habit of *S. sanguinolenta* from which it differs by its dimorphous leaves—

\* As reported in several other species by Campbell [6], p. 530, Eames [13], p. 34, and Gibson [21], p. 453.

\*\* In India the altitude is represented by feet; approximately 1000ft=304m, 5000ft=1524m, 10000ft=3048m, 15000=4572m.

1. Baker [2]. 2. Calc. Herb. 3. Engler-Prantl [15]. 4. Spring [45]. 5. Calc. Herb.; Spring [45]. 6. Uphof [47], p. 105; Baker [2]; Calc. Herb. 7. Engler-Prantl [15]; Spring [45]. 8. Baker [2]; Uphof [47] p. 105. 9. Dehra Dun Forest Herb. 10. Alston [1] (only additional localities incorporated). 11. Prof. M.O.P. Iyengar coll.



Baker, [2], p. 74). *Eastern Himalayas*<sup>7</sup>, *Western Himalayas*<sup>3</sup>, *Kashmir*<sup>3,4</sup>: Banhatti<sup>2</sup>, 2952 m; Pahlgam<sup>12</sup>; Mt. Pendijegam<sup>4</sup>. *Panjab*: Lahul, 11000 ft. (dry rock crevices)<sup>12</sup>; Simla<sup>12</sup>. *W. Pakistan*: Siran<sup>12</sup>; Sind and Chenab valleys<sup>12</sup>. *Afganistan*<sup>12</sup>: Kurram<sup>12</sup>.

f) *S. adunca*, A. Br. (confined to the Western Himalayas). *N. W. Himalayas*<sup>2</sup>: (coll. Tucker)<sup>2</sup>; Simla<sup>2</sup> (7000 ft. Tucker)<sup>12</sup>. *U. P.*: Jansaar Dt., 7000 ft. (coll. Gamble)<sup>2</sup>; Tehri Garhwal<sup>3</sup> (coll. J. S. Gamble, 27214)<sup>2</sup>; Kumaon (coll. H. G. Champion, 7803)<sup>13</sup>; Mussurie<sup>2</sup>, Chakrata Road<sup>13</sup>, Aglar valley (coll. Duthie-1877, 7)<sup>13</sup>; Garhwal (Stewart)<sup>12</sup>, Churani (coll. G. A. Gammie)<sup>13</sup>, near Ringali Gadh, Dehra Dun (Kalsi)<sup>12</sup>; Kashmir<sup>12</sup>.

g) *S. bryopteris*, Bak. (*S. tamariscina* (P. B. Spr.) Alston). (Central and Peninsular India at low elevations). *Ostindien*<sup>3</sup>. *Tibet*: Khamba La<sup>2</sup>, 16000 ft. (coll. Capt. H. G. Walton, and Major F. E. Younghusband, 1904)<sup>2</sup>. *U. P.*: On sandstone hills, Meja Tehsil, Allahabad (coll. Anderson)<sup>12</sup>; Sandstone rocks, Patha, Banda (coll. T. Edgeworth)<sup>12</sup>; On low hills near Benares (coll. Mehta)<sup>12</sup>; Banda (Karvi)<sup>6</sup>; Arjar, Bundelkhund (coll. J. F. Duthie, 6605)<sup>13</sup>. *Bihar*<sup>4</sup>: (coll. Hamilton)<sup>4</sup>; Rajgir (coll. R. S. Rao<sup>11</sup>), Rajahra<sup>6</sup> (coll. Sinha)<sup>6</sup>; Bihar (coll. Roxburgh)<sup>12</sup>; Dhoo Khond near Sasaram, 500 ft. (coll. Levinge)<sup>12</sup>; Roridee, Lohardaga (coll. Clarke, 34319)<sup>12</sup>; Singbhum (dry forests, Haines)<sup>14</sup>. *Central and Peninsular India*<sup>1</sup>: Anandgarh<sup>10</sup> (Rewa)<sup>10</sup>; Bowtee Fall, Rewa (coll. Banthaud)<sup>12</sup>; Near Khandwa (coll. J. F. Duthie, 8348)<sup>13</sup>; *S. India*<sup>4</sup>.

h) *S. involvens*, (Sw.) Spr. *Ostindien*<sup>3</sup>. *Eastern Himalayas*<sup>7</sup>. *Assam*: Mawnluh, 4500 ft. on rocks (coll. N. L. Bor, 199, 209)<sup>13</sup>. *U. P.*: Kumaon (coll. Davinder)<sup>13</sup>; Kamoon (coll. Wallich)<sup>4</sup>; Almora (coll. Jameson)<sup>13</sup>; Mussoorie (coll. Jameson)<sup>13</sup>; Kali valley, Askot (coll. J. F. Duthie)<sup>13</sup>. *Peninsular India*<sup>4</sup>.

i) *S. caulescens*, (Wall.) Spr. (India, Burma, Ceylon). (Final branchlets curl up on drought: Bak. 1887, p. 85). Ascending to 7000 ft. in Nepal. *Bengal*: Darjeeling (coll. B. L. Gupta)<sup>12</sup>. *Assam*: Janak mukh<sup>2</sup> (coll. Burkill, 37274)<sup>2</sup>; Manipur<sup>3</sup> (Sirohipurar)<sup>2</sup>; Khasi hills<sup>12</sup>; Cherrapunji<sup>12</sup>; Lushai hills<sup>12</sup>; Abor hills<sup>12</sup> (coll. Kingdonward)<sup>12</sup>; Senchal<sup>12</sup>; Kohima<sup>13</sup>, 4000 ft., Naga hills<sup>13</sup> (coll. N. L. Bor, 15658)<sup>13</sup>. *Bhutan*: Rocha chu valley<sup>12</sup>; Trashiyangsi<sup>12</sup>. *Nepal*: Narain hetty<sup>12</sup>; Burghoo (7000 ft.)<sup>12</sup>. *U. P.*: Near Pithoragarh (5000 ft.)<sup>12</sup>; Askot<sup>12</sup>, 4-5000 ft. (coll. Duthie, 6309)<sup>12,13</sup>; Dhauli valley (5-6000 ft.)<sup>12</sup>; Kali valley (3-4000 ft.)<sup>12</sup>; Kumaon<sup>8</sup> (coll. Duthie)<sup>8</sup>; Mussoorie<sup>12</sup>; Almora<sup>12</sup>. *S. India*<sup>2</sup>: Malabar<sup>2</sup> (coll. T. Anderson)<sup>2</sup>; Nilgiris<sup>12</sup>; Kutallam<sup>12</sup>; Kotagiri<sup>12</sup>; Pykara falls (6000 ft.)<sup>12</sup>; Palnis<sup>12</sup>; Kodaikanal<sup>12</sup>; Walaghat (Sispara)<sup>12</sup>. *Ceylon*<sup>1</sup>: Ramboda<sup>12</sup>; Dankande Pass<sup>12</sup>; Matale<sup>12</sup>. *Burma*: (coll. Kurz)<sup>2</sup>; Dt. Meiktila<sup>9</sup>; Taungbaw-yo<sup>9</sup> (coll. H. C. Smith)<sup>9</sup>; Pegu<sup>12</sup>; Tavoy<sup>12</sup>; Mogok<sup>12</sup>.

j) *S. concinna*, Spr. *Ceylon*<sup>4</sup>: (coll. Wallich, Hooker)<sup>4</sup>. Spring's Neilgherry and Ceylon specimens are probably *S. plumosa*. Baker (1887), p. 52.

k) *S. chrysocaulos*, Hk. and Gr. (Principally Northern Indian mountains). *Eastern and Central Himalayas*<sup>1</sup>. *Northern India*<sup>4</sup>. *Bengal*: Darjeeling<sup>12</sup> (coll. B.

1. Baker [2]. 2. Calc. Herb. 3. Engler-Prantl [15]. 4. Spring [45]. 5. Prof. B. Sahni coll. (S.K. Pande, 1929). 6. Sinha [44]. 7. Baker [2]; Uphof [47], p. 105. 8. Baker [2]; Calc. Herb. 9. Forest Herb., Maymyo, Burma. 10. Dr. S. C. Verma found the species growing on rocky slopes. 11. B.S.I. excursion coll. 12. Alston [1] (only additional localities incorporated). 13. Dehra Dun Forest Herb. 14. Haines [26], p. 225.

L. Gupta<sup>5</sup>. *Assam*<sup>2</sup>: Khasia hills<sup>2</sup>; Shillong peak<sup>6</sup>; Rangkhengsning<sup>6</sup> (coll. Kanjilal)<sup>5</sup>. *Bhutan*: Trashiyangtse valley<sup>6</sup>, 7500 ft. (coll. Ludlow and Sherriff)<sup>6</sup>. *Sikkim*: Phadenchen<sup>2</sup>, 8000 ft. (coll. W. W. Smith)<sup>2</sup>. *Nepal*: Burghoo (6-7000 ft.)<sup>6</sup>. *Kashmir*: Basoli<sup>5</sup>, 6000 ft. (coll. Clarke, 31610)<sup>6</sup>. *U. P.*: Kumaon (7-8000 ft.)<sup>1</sup>; Mussoorie<sup>6,7</sup> (coll. R. M. Gorie<sup>6</sup>, 3993; H. Hooker<sup>3</sup>); Dehra Dun<sup>6</sup> (coll. J. F. Duthie; R. N. Parker<sup>6</sup>); Naini Tal<sup>6</sup>, Agarpatta, China peak (coll. H. G. Champion)<sup>6</sup>; Sylhet at Kumaon (coll. Wallich; H. Hooker)<sup>3</sup>; Nainital (7000 ft.)<sup>6</sup>. *Panjab*: Simla<sup>2</sup> (coll. R. N. Parker<sup>6</sup>, 63695; Lady Dalhousie<sup>3</sup>; Hooker<sup>3</sup>); Elysium hills (Simla)<sup>6</sup>; Dalhousie<sup>6</sup>; Kalatop forest (Chamba)<sup>6</sup>. *S. India*<sup>3</sup>: Madras<sup>3</sup> (coll. Wight; Lady Bentinck).

l) *S. chrysorhizos*, Spr. *Assam*<sup>1</sup>: (coll. Griffith; Hooker)<sup>3</sup>; Khasia hills<sup>1</sup>; Gauhati hills<sup>5</sup>; Sylhet<sup>5</sup>. *U. P.*: Mussoorie<sup>4</sup>; Garhwal<sup>3</sup> (coll. Griffith; Hooker)<sup>3</sup>. *Burma*; Toungoo<sup>6</sup> (coll. C. O'Riley)<sup>6</sup>.

m) *S. repanda*, (Desv.) Spr., Alston. (*India*, *Nepal*, *Burma*). *Bengal*: Chandernagore<sup>2</sup> (coll. Hosain)<sup>2</sup>. *Nepal*<sup>2</sup>: (coll. C. Maries)<sup>2</sup>. *Bihar*: Parasnath<sup>2</sup>, 2300 ft. (coll. D. Prain)<sup>2</sup>; Hundrugarh<sup>2</sup>, Chota Nagpure<sup>2</sup> (coll. Prain)<sup>2</sup>; Hazaribagh<sup>5</sup>; Monghyr<sup>5</sup>; Rajmahal hills<sup>5</sup> (coll. Wallich)<sup>5</sup>. *Orissa*: Kapilas hill (1000 ft.)<sup>6</sup>, Dhen Kanal state<sup>6</sup> (coll. H. F. Mooney, 2722)<sup>6</sup>; Balasore hills<sup>5</sup>; Bastar state<sup>6</sup>, Bailadila<sup>6</sup> (coll. H. F. Mooney)<sup>6</sup>. *U. P.*: Nainital<sup>6</sup> (coll. H. G. Champion<sup>6</sup>, 10825). *M. P.*: Khandwa<sup>6</sup>; Joshpur<sup>6</sup>. *S. India*: Chandragiri (Ganjam)<sup>6</sup>; Rampa (Godavari)<sup>6</sup>. *Burma*<sup>2</sup>: Tauch<sup>2</sup>; Kadu hills<sup>5</sup>; Ava<sup>6</sup>; Mamy<sup>6</sup>; Mergui<sup>6</sup>; Mt. Popa<sup>6</sup>.

n) *S. imbricata*, Roxb. (?). *U. P.*: Patha (Banda), on rocks. coll. Edgeworth, [14], p. 321.

## THEORETICAL

The clustering or curling movement of the branches of *S. bryopteris* is purely hygroscopic and is shown by the fact that they easily uncurl and regain their original shape when moistened and that the one or the other condition may be induced at will according as water is added or withdrawn [29]. Uphof [47] holds the view that the movements of the tissues in the resurrection species are entirely mechanical as their cells when old do not contain living protoplasm even in living plants; moreover similar results are obtained in dead plants; they depend upon the hygroscopic capacities of the tissues and are entirely physical instead of biological.

According to Jost [29] the bending and twisting of hygroscopic organs are obviously produced only if the capacity for swelling varies in different directions, where layers having great powers of imbibition stand in antagonism to those with less capacity for swelling. Haberlandt [23] remarked that it is the difference in the power of imbibition in different tangential planes which are of great importance, the active expansion and contraction of the walls may take place either in the transverse or in the longitudinal direction; the hygroscopic swelling and shrivelling of cell walls involves changes in the volume of the

1. Baker [2]. 2. Calc. Herb. 3. Spring [45]. 4. Prof. B. Sahni collection. 5. Alston [1]. (only additional localities incorporated). 6. Dehra Dun Forest Herb. 7. Prof. B. Sahni collection; Spring [45].

membranes concerned, which under suitable conditions may produce a very appreciable amount of movement *in the shape of a curvature or torsion of some limited portion of the plant body*. It is exactly what happens in the curling branches of *S. bryopteris*.

Leclerc du Sablon [32]\* thought that the curling movement in *S. lepidophylla* is due to a difference in the cell walls on the two sides of the stem and found that the upper side is thick-walled and the lower thin-walled. Wojnowic [52]\* also found the same thing and added that the thick-walled cells absorb more water than the thin-walled ones and the former lose more water during drought than the latter, causing the plant to curl up and to have the appearance of a cluster ball. I also observed similar construction in the cortical layers of *S. bryopteris* in its upper curling stem. As already described the upper epidermis is followed by 2-3 layers of thick-walled cells (hypodermis) with narrow lumina which are followed by a zone of very thick-walled, well stretched, turgescient, large cavitated cells comprising the hygroscopic tissue with great power of contraction and expansion. That these cortical cells of the upper (dorsal) side of the stem are definitely thicker-walled than the cells of the lower (ventral) side is shown by Table 1 (Figs. 8, 9). Ventrally a 3-4 layered zone of thin-walled parenchymatous cells immediately beneath the stele is followed by a peripheral band of sclerenchyma which serves as a strengthening flange to withstand the longitudinal stretch caused by the contraction of the hygroscopic dorsal cells. Such an ag-

TABLE 1

The table shows in micrometer divisions the thickness of the wall and the diameter in the widest axis of the lumina of the cortical cells of the stem at the curling region under a magnification of ca. 2000. The figures are based on an average of 10 readings in each. The figures within bracket indicate the range.

Number of observation	Part of the cell	Dorsal cortex		Ventral cortex	
		Motor cells (outer cortex)	Parenchymatous cells of inner cortex	Parenchymatous cells of inner cortex	Outer sclerenchymatous flange
I	Wall (thickness)	4.5 (4-5)	2 (1-3)	2 (1.5-3)	5 (4-5.5)
	Lumina (diameter)	19 (17-23)	12 (10-13)	14 (12-15)	6 (2-7)
II	Wall (thickness)	5 (4.5-5.5)	1.9 (1.5-3)	1.9 (1-3)	5.5 (5-6)
	Lumina (diameter)	18 (17-20)	14 (11-17)	16 (12-20)	8 (5-10)
	Remarks	Very wide stretched and thick-walled cells (wide zone)	Quite wide stretched and thin-walled cells (narrow zone)	Quite wide stretched and thin-walled cells (wide zone)	Very narrow and very thick-walled cells (narrow zone)

\* As cited by Uphof [47].



gregation of the mechanical cells into a single compact and solid mass is the most advantageous arrangement for organ which has to withstand longitudinal tension as has been asserted by Haberlandt [23]. Further the tubular spaces (Fig. 8) which occupy the corners of the hygroscopic 'motor cells' obviously allow for the dilation of these cells when in fully turgid and expanded state.

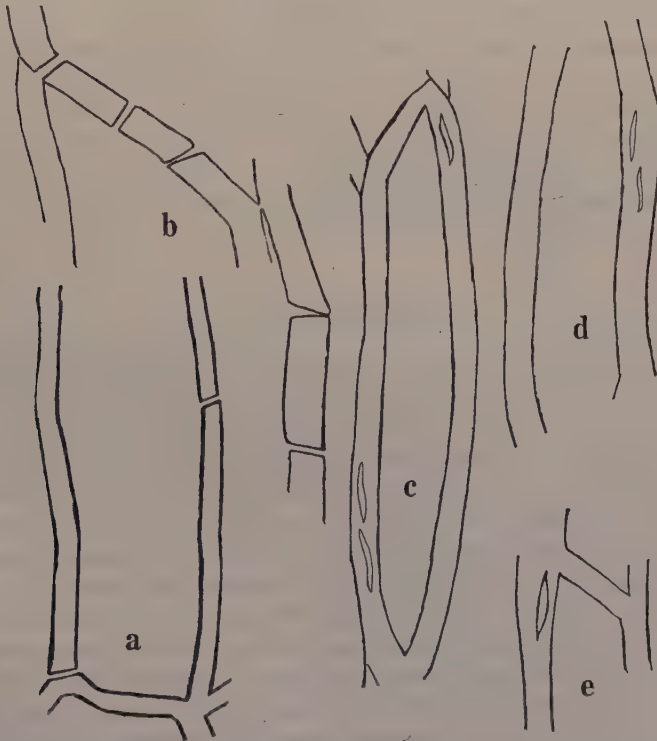
A certain degree of analogy is to be seen in the case of a tendril as the coils are pulled apart the concave face is stretched and its convex face compressed [23]. Such a pull is always experienced by the natural weight of the plant by such external disturbances as strong wind or animal agencies. Hence the mechanical tissue must be strongly developed as a 'tension flange' [23] on the concave side of the coiled region which in this case is subjected to the stretching force. The convex side which experiences the compression provides the effective 'compression flange' [23] in the form of the turgescent parenchyma comparable to the turgescent 'motor cells' of the upper cortex of *S. bryopteris*. Thus the turgescent tissue is effective for compression and the mechanical strengthening zone is to withstand stretching. Similarly the subepidermal fibrous strands on the abaxial side of the leaves of many xerophilous grasses which roll in on desiccation and the mechanical system of a clasping leaf-sheath with its fibrous strands well developed beneath the abaxial epidermis, have the same function of a tension flange. Tschirch\* even regarded the abaxial fibrous strands as the active components for rolling and thought that the fibrous layers have a greater power of imbibition and hence a greater tendency to contract on drying than the more superficial layers. The rolling of leaves, however, is not strictly comparable to the curling of the twigs of *Selaginella* in the sense, that as explained by Steinbrinck\* and also thought by Haberland [23] that the former movement is a cohesive one, whereas the latter is hygroscopic. These instances clearly demonstrate the fact that the disposition of fibrous strands is solely determined by mechanical considerations.

A longitudinal section through the curling region in *S. bryopteris* reveals that the so-called hygroscopic motor cells below the dorsal hypodermis are prosenchymatous with a few transverse pittings (on dorsal and ventral surfaces), whereas the ventral thin-walled cells, just beneath the stele, are parenchymatous and have simple, slightly oblique pittings (Text-fig. 2). The lowermost peripheral sclerenchyma band (tension flange) is composed of lignified obliquely pitted thick-walled cells. This difference in the disposition of pitting is possibly effective in causing the curvature. The antagonism between the two sides of the hygroscopic organs is due to their being composed of different tissues. The bending, twining and twisting of hygroscopic organs can obviously be produced only if the capacity for swelling varies in different directions, when layers with great powers of imbibition stand in antagonism to those with less capacity for swelling. A striking analogy is seen in the movements of the involucre of bracts of the ripe inflorescence of *Centaurea* and certain other Compositae, which curl inwards in damp (to prevent the escape of the wind dispersed fruits

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\* As cited by Haberlandt [23].

during rainy weather) and outwards in dry air. Steinbrinck observed in these bracts there is situated immediately beneath the outer surface which on drying becomes the concave side, comparable to the dorsal cortex of the present species of *Selaginella* stem a layer of prosenchymatous cells with transversely elongated pits. This layer therefore swells and contracts more actively in the longitudinal than in the transverse direction. The layer inner to this is parenchymatous in character; its walls are also transversely pitted but have a smaller power of imbibition than those of the first layer. The third layer following this inner layer is likewise parenchymatous but has somewhat oblique pits. Next to the inner surface of the bract (which becomes the convex side on drying) there is a second prosenchymatous layer (comparable to the ventral sclerenchymatic flange of the stem of *S. bryopteris*) with very oblique to longitudinal pits [23], a feature which shows it to be the component antagonistic to the outermost layer. The axes of the maximum contraction of the two prosenchymatous layers thus intersect one another at right angles. On drying the outermost layer undergoes the greatest, the innermost the smallest amount of contraction in the longitudinal



Text-fig. 2. a-b, L. S. of 'motor cells' showing transverse pittings which are cut at right angles. c-e. L. S. of sclerenchymatous cells of the ventral 'tension flange' showing oblique pittings. × ca. 2000

direction and that whole bract consequently curls outwards [23]. The branches of the familiar Rose of Jericho (*Anastatica hierochuntica*) which curl inwards when dry and outwards when wet have a similar mechanism. According to Steinbrinck in the *adaxial* half of each branch, the fibrous motor cells (cf. similar cells in *S. bryopteris*) are transversely pitted, while the corresponding elements in the *abaxial* half (cf. sclerenchymatous flange of the stem of *S. bryopteris*) have very oblique pits; the antagonism is therefore of the same nature as in *Centaurea*. The folding mechanism, in dry weather, of the fan-shaped lamina of *Actiniopteris radiata* [38], a xerophytic fern of the region S. Africa to India, forms another example of such movement.

Thus it is interesting to see that such unrelated plants will show in their different organs\* similar structural mechanism for performing the identical function of withstanding unfavourable condition of drought. The effect of environmental physical conditions tell similarly upon the internal structures of such unrelated life forms. Such epharmonic adaptations are merely reflections as truly as possible of the prevailing conditions of life and cause plants otherwise systematically placed widely apart to look extraordinarily alike one another in regard to the structural features (external or internal) of the vegetation shoot [5, 51]. Lastly it may be said that the curling movement in the branches of *S. bryopteris* appears to be also considerably aided by the drawing in of the expanded sclerotic margins of the ventral leaves, which lie overlapping one another immediately beneath the branches. In dry conditions these sclerotic wings of the leaves clasp strongly round the stem and press it hard to bend it in. It may also be mentioned that Gibson [20] remarked that the inrolling habit of *S. pilifera*, *S. lepidophylla* and *S. involvens* may have something to do with the equivalence of the epidermis on both leaf surfaces.

## SUMMARY

The present paper describes for the first time a xerophytic species of *Selaginella* e. g., *S. bryopteris*, Bak. from India. The general morphology, ecological anatomy and the distribution of fourteen definitely known xerophytic species of *Selaginella* in India, Pakistan, Burma and Ceylon have been studied.

*S. bryopteris* has been recorded from Karvi (Banda, U.P.), Rajahra (Bihar), Anandgarh (Central India) and Peninsular India and Tibet. It has the character of inrolling its branches during drought like *S. lepidophylla*. The plants are erect 5-10 cm high with lower simple unbranched and upper much branched deltoid stem (cf. *S. imbricata*). The lower part is covered with a few scaly leaves but the upper region bears four rows of dimorphic leaves, the dorsal ones being ascending oblique ovate, the ventral ones much imbricated, unequal sided, serrate and more or less adpressed on the sides of the stem. The older portion of the stem, near the basal region, has strongly sclerenchymatous cortex and a large semicircular mass of stelar tracheids with 3-4 marginal protoxylem groups and a narrow lacuna filled with loosely packed cells. The upper branches show

\* Tendril, bract, stem and branches.



marked dorsiventrality where the cortex of dorsal side is mainly composed of thick-walled, large, turgid, hygroscopic 'motor cells' capable of expansion and contraction; the ventral cortex is thin-walled, parenchymatous and is flanked underneath by a layer of sclerenchyma which evidently serves as a 'tension flange' to withstand the pull exerted by the dorsally incurving stem during drought. The dorsiventrally flattened ribbon-like monostele consisting of a central mass of tracheids with two marginal protoxylem groups, 2-3 layers of phloem parenchyma, a layer of flattened sieve tubes (imperfectly developed opposite protoxylems), a few protophloem elements and a layer or two of chlorophyll containing pericycle, is held in the lacuna by trabeculae composed of a tubular endodermal and one or two inner cortical cells. The stem is protostelic throughout. The rhizophores seem to be absent as in *S. cuspidata*, *S. lepidophylla*, *S. involvens* and *S. pilifera*. The roots have a prominent covering of root hairs and the usual monarch stele. The leaves possess a thick deposition of shiny warty cuticle on both epidermises, strong hypodermis, a well developed double layered palisade (cf. *S. Lyallii*) on the surface exposed to sun, sclerotic margins shielding the stem, and a concentric vascular bundle. The quadrangular cone with keeled sporophylls, two layered (persisting tapetum) sporangium wall, numerous microspores and three megaspores has a thin stele supported by long, 2-3 celled trabeculae. The apex of the cone is occupied by a two sided wedge shaped apical cell as in *S. Martensii*.

The mechanism of the curling movement of the stem is discussed. The movement which is purely hygroscopic seems to be caused by unequal power of swelling of the different tissues (e. g. the upper turgescient 'motor cells', the lower parenchyma and the sclerenchymatous flange), augmented by the peculiarities of pittings on their walls and is comparable to the mechanism seen in Rose of Jericho.

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I am deeply grateful to the late Prof. B. Sahni, who had kindly entrusted this piece of work to me, for his valuable suggestions and criticisms during the course of this work and for his kind permission to use his personal library. I owe a high debt of gratitude to Professor Y. Ogura of the University of Tokyo for kindly going through the manuscript and giving me the benefit of his valuable criticisms. My thanks are also due to the authorities of the herbaria of the Royal Botanic Gardens, Calcutta and the Forest Research Institute, Dehra Dun for kindly placing at my disposal for examination all the sheets of *Selaginella*. I wish also to express my indebtedness to my friend the late Mr. R. N. Misra, Lucknow University, for help in the microphotographic work. Finally to the authorities of the University of Lucknow I am indebted for the award of a Research Fellowship to me during the course of this work. At the end I am thankful to Prof. P. Maheshwari for affording me the privilege of access to his personal library.

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### Explanation of Plates

(*Selaginella bryopteris* (L.) Baker)

#### Plate I

Fig. 1. T. S. of stem (older, basal region). o. c.=outer cortex; m. c.=middle cortex



(sclerenchymatous); i. c.=inner cortex; s. t.=sieve tube area; xy.=xylem mass with marginal protoxylem groups (shaded); (outline figure). per.=pericycle; phl. par.=phloem parenchyma. × ca. 32

Fig. 2. The stelar region (magnified). tr.=trabeculae; per.=pericycle; pt.=proto-phloem; s. t.=sieve tubes; phl. par.=phloem parenchyma. The central semicircular mass of tracheids with protoxylem groups (p. xy.). × ca. 200

Fig. 3. T. S. of stem (upper curling region). ep.=epidermis; d. o. c.=dorsal outer cortex of hygroscopic motor cells; i. c.=inner cortex of thin-walled cells; v. o. c.=ventral sclerenchymatous outer cortex forming the 'tension flange'; t.=trabeculae. × ca. 180

Fig. 4. L. S. through cone apex. a.=apical cell. × ca. 400

Fig. 5. The dorsal leaf. c.=cusp. × ca. 25

Fig. 6. The ventral leaf. scl. sh.=sclerotic sheet. × ca. 32

Fig. 7. The sclerotic sheet of the ventral leaf, which clasps the stem from beneath. scl. sh.=sclerotic sheet. × ca. 400

### Plate II

Fig. 8. T. S. of 'motor cells'. t. s.=tubular spaces. × ca. 800

Fig. 9. T. S. of thin-walled cortical cells. × ca. 800

Fig. 10. T. S. of sclerenchymatous cells of the ventral 'tension flange'. × ca. 800

Fig. 11. T. S. of a dorsal leaf. e.=epidermis; pal.=palisade; r. m.=reticulate mesophyll; scl.=sclerotic margin of the leaf; strong hypodermis also seen. × ca. 280

Fig. 12. A magnified view of a portion of above. l. t.=leaf trace; other explanations as in Fig. 11. × ca. 800

Fig. 13. T. S. of ventral leaf. scl. sh.=sclerotic sheet; w.=warty cuticle; other explanations as in Fig. 11. × ca. 180

Fig. 14. T. S. through the sclerotic sheet terminating on the margin to a single cell thickness. × ca. 440

### Plate III

Figs. 15-17. Plants showing the lower erect portion and upper inrolled branches (during drought). × ca. 3/5

Fig. 18. Showing shape and arrangement of leaves in the upper branches. a-b=dorsal view; c-d=ventral view. × ca. 4/5

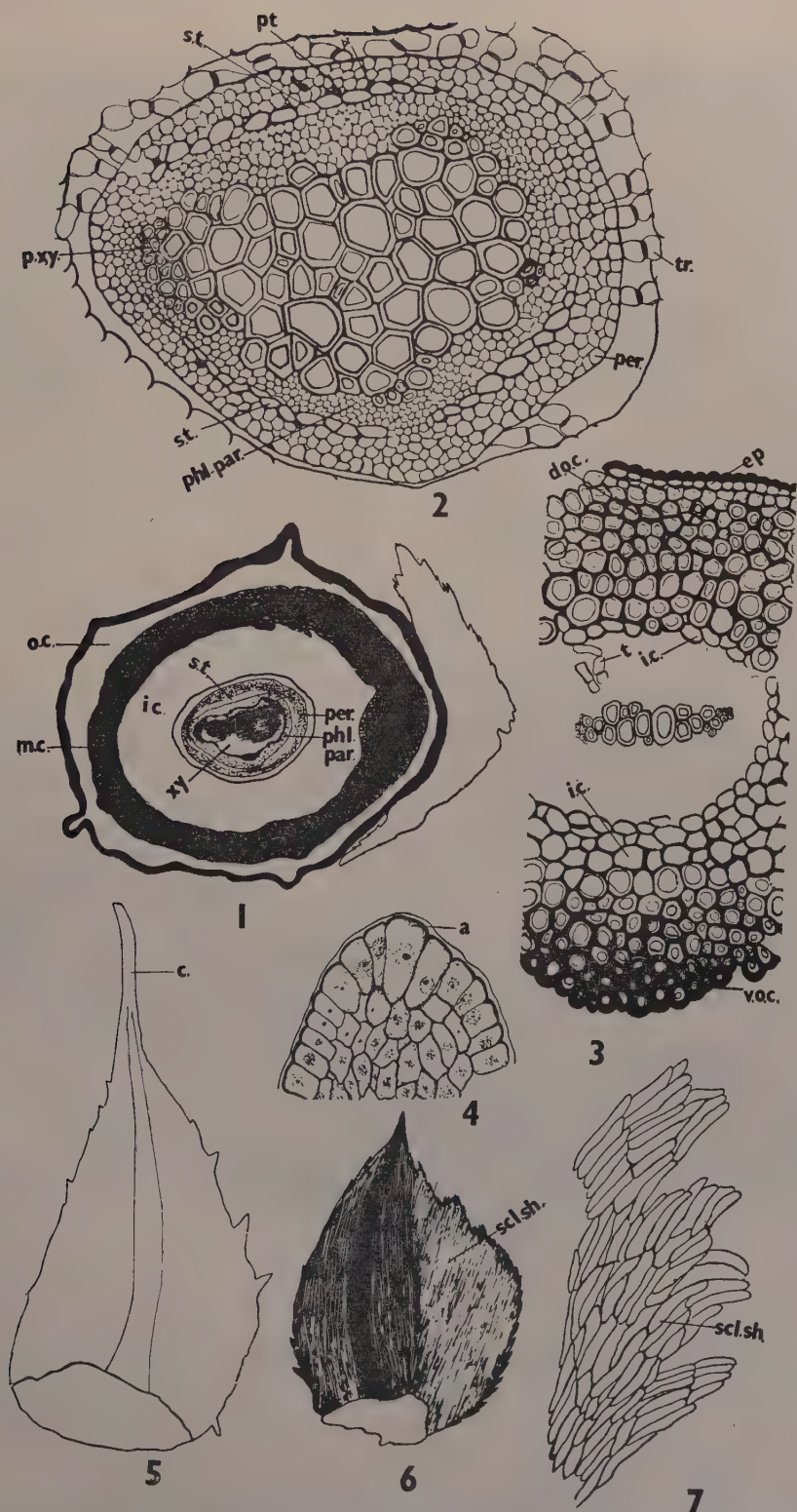
Fig. 19. T. S. of the stem near the apex, showing the arrangement (disposition) of the dorsal (d. l.) and ventral (v. l.) leaves. The aligular surface of the dorsal leaves and the ligular surface of the ventral leaves show similar anatomical structure and vice versa. × ca. 135

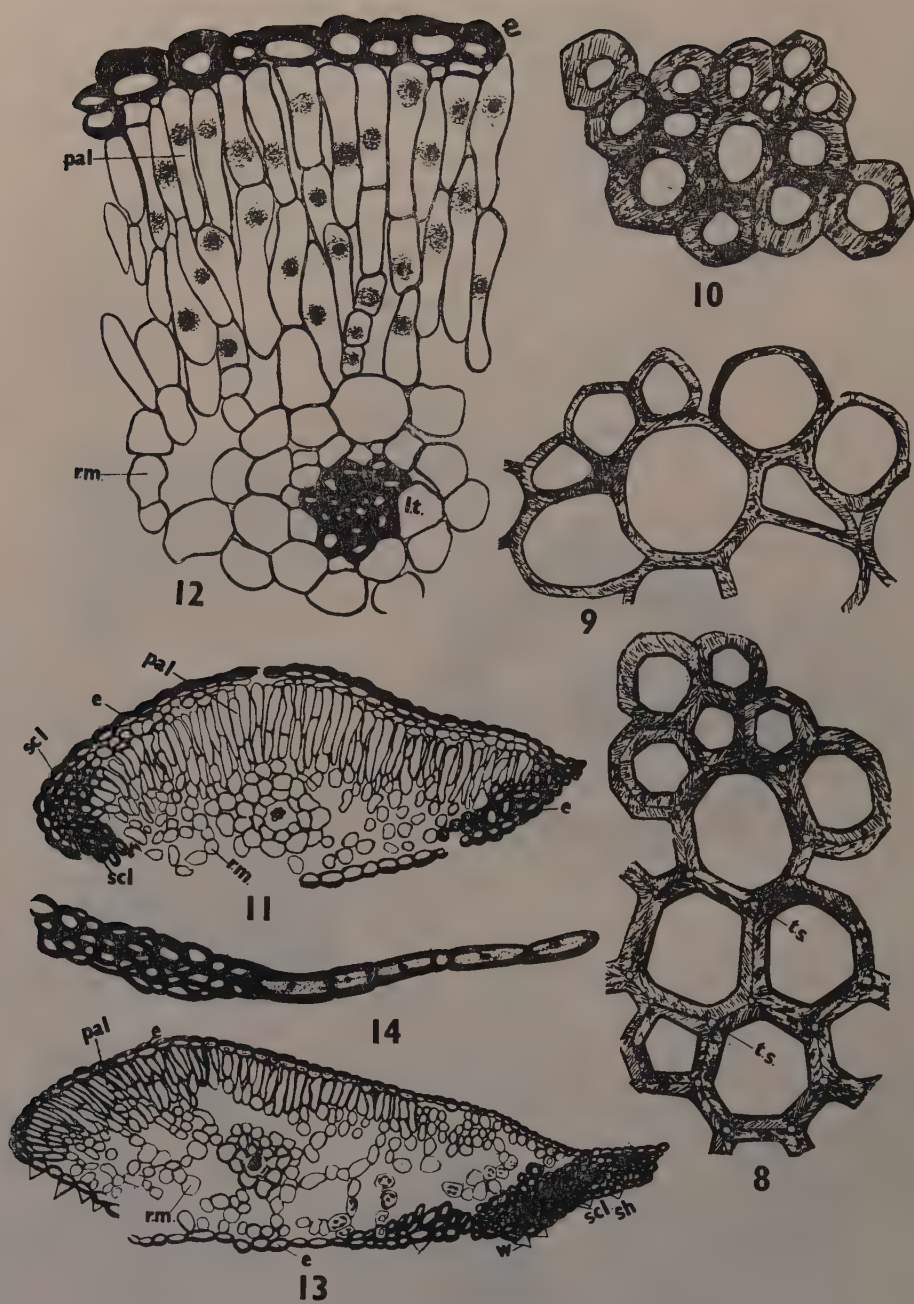
Fig. 20. Roots. × ca. 4/5

Fig. 21. T. S. of root. Note the piliferous layer (p.) and the crowing root hairs (h.). o. c.=outer cortex; m. c.=middle cortex; i. c.=inner cortex; e.=endodermis. Note the dark phloem region almost surrounding the monarch xylem. × ca. 75

Fig. 22. L. S. through the inrolling stem. The left side is the dorsal region; note the thick-walled motor cells. The tracheidal (end walls seen) stele running through the lacuna is held by trabeculae. The ventral cortex composed of thinner-walled cells and the ventral peripheral flange of thick-walled sclerenchymatous (appearing as dark band in the photograph, scl. fl.) cells and the clasping sclerotic wings (s. w.) of the ventral leaves are well seen. × ca. 45

Fig. 23. T. S. of stem through curling region. The thick-walled hygroscopic cells of the dorsal cortex, the innermost layer of thin-walled cells in the dorsal cortex, the thin-walled ventral cortical cells and the peripheral sclerenchymatous band on the ventral



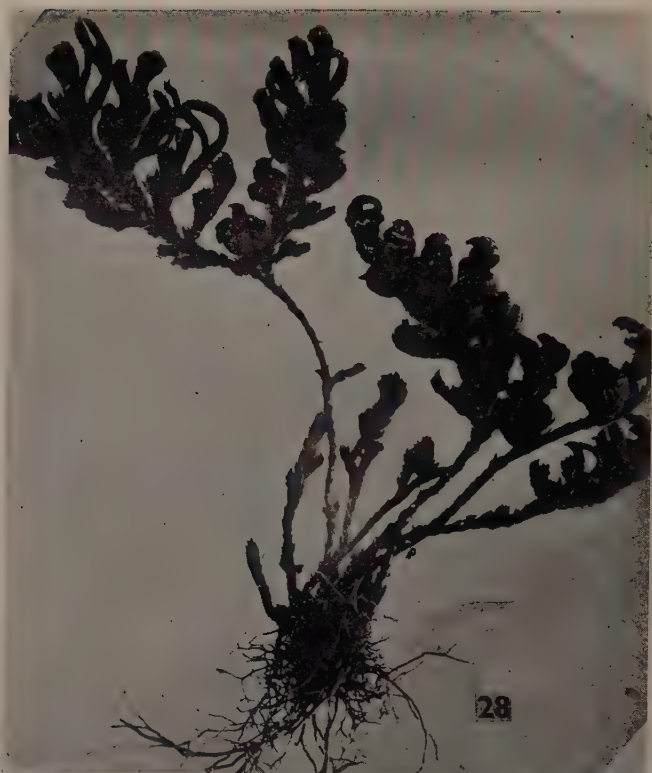


CHOWDHURY, N. P.: Structure and ecology of xerophytic *Selaginella* from India.





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surface are seen. The trabeculae in the lacuna and the dorsiventral stele with 2-3 protoxylem groups are also seen. Details as in Fig. 29. × ca. 80

Fig. 24. T. S. through the lower region of the cone. mc. sp.=microsporangium with microspores; mg. sp.=megasporangium with megaspores. Note the cubical thick-walled cells of the sporangium wall (sp. w.), a second layer of presisting tapetum. The central stele is situated in the lacuna. × ca. 100

Fig. 25. T. S. of the cone stele. Note the well developed lacuna, the 3-celled trabeculae (tr.), a conspicuous layer of chlorophyll containing pericycle (p.) and the central stele. phl. p.=phloem parenchyma; xy.=tracheids; p. xy.=protoxylem; c. w.=cutinized wall of the endodermis (e.) cell. × ca. 220

Fig. 26. L. S. through the stem apex. a. c.=wedge shaped apical cell. × ca. 550

Fig. 27. T. S. of a dorsal leaf. The elongated epidermal cells, the warty cuticle (w.), the double layer of palisade (pal.) and the lower spongy mesophyll (s. m.) are seen. The vascular bundle is mesarch concentric. × ca. 450

#### Plate IV

Fig. 28. A xerophytic specimen collected from Banda (U. P.). The lower erect portion of the stem is covered with scaly leaves, the upper region is branched and bears dimorphic leaves. The curled branchlets on the right illustrate the inrolling character of the plant. The roots are well developed. × ca. 1½

Fig. 29. T. S. through the upper curling region of the stem, showing marked dorsiventrality in the structure. The dorsal cortical hygroscopic motor cells (mt. c.) are definitely thicker-walled than the ventral cortical cells (v. c.). The ventral peripheral flange of the strengthening tissues (scl. fl.) is composed of lignified sclerenchymatous cells. Note the double layer of palisade tissue in the attached dorsal leaf and a portion of the sclerenchymatous wing (s. w.) of the ventral leaf, clasping the stem from beneath. The lacuna, trabeculae and the dorsiventral ribbon-like stele with two marginal protoxylem groups are seen. × ca. 200



## ECOLOGICAL STUDIES ON INTERSPECIFIC COMPETITION IN A PLANT COMMUNITY I.

### AN ANALYSIS OF GROWTH OF COMPETING PLANTS IN MIXED STANDS OF BUCKWHEAT AND GREEN GRAMS

HIDEO IWAKI\*

#### INTRODUCTION

The principal phytosociological factor in plant succession and the geographical distribution of plant species is naturally the interspecific competition, which selects the plant species that can survive in nature after severe and long struggle with other species. Therefore, many efforts of biologists as well as of ecologists have been devoted to elucidation of this problem: the work of Clements, Weaver and Hanson [3] is one of the classics in this line. Recently the problem has also been studied by the number of agriculturists with special reference to weed control in crop fields [4, 7, 8].

These studies have provided much information as to the final results of interspecific competition under various ecological and phytosociological conditions. However, as yet only a few have been concerned with the working mechanisms which operate in the competition process. Kramer and Decker [6] have studied the competition between pine and oak in a piedmont forest with reference to the production of matter in these species, and concluded that the pine fails in the competition because of the heavy reduction of its photosynthesis in the shade. Besides the importance of photosynthetic activity, Walter [11] has pointed out that the proportion of photosynthate distributed into the photosynthetic organs (leaves) and non-photosynthetic ones (stems, roots, etc.) might be the factor of primary importance in determining the final result of competition. A theoretical analysis based upon the production of matter of interspecific competition in the plant succession process was presented by Monsi and Oshima [9].

Although the inherent ability of species in the production of matter is a very important character in competition among plant species, besides this the growth in height itself should be taken into account in connection with struggle for light, as Boysen-Jensen [2] has pointed out, because the higher the plant is, the more light will be received by it, accompanying the higher production of matter and interception of light from the lower plants. Monsi and Saeki [10] have shown in the seasonal development of the productive structure of grassland communities in central Japan, that the development of the lower synusium, of *Sanguisorba*, *Euphorbia*, or *Thalictrum*, is generally limited by the light intensity which is decided by the leaf amount of the upper synusium, of *Phragmites* and *Miscanthus*.

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In the present study, the author designed a series of mixed-stand experiments of two crop plants, buckwheat and green grams, in the most fundamental form of interspecific competition, intending to make clear the ecological significance of height growth difference, since these two species have almost the same dry matter productivity. Also the growth analysis of the competing plants was carried out, on the basis of dry matter production computed from the photosynthesis under a given light condition, the respiration, and the distribution of photosynthate into photosynthetic and non-photosynthetic organs.

#### EXPERIMENT A. SYNCHRONOUS MIXED PLANTING OF BUCKWHEAT AND GREEN GRAMS

##### *Methods*

Field experiments were carried out in the experimental field of the Faculty of Science of the Tokyo University of Education, at Hōya, in the suburbs of Tokyo. Prior to the sowing of seeds, barnyard manure and chemical fertilizer were applied to the soil in sufficient quantity to minimize the competition for nutrient salts. After such preliminary preparation of the soil, no further application of fertilizer was made.

As the experimental material, buckwheat (*Fagopyrum esculentum*) and green grams (*Phaseolus viridissimus*) were employed. The experimental area was divided into the following three plots, each of which was 10 m. square: a pure stand of buckwheat (A-1), a pure stand of green grams (A-2), and a mixed stand of buckwheat and green grams (A-3).

In this experiment, all the seeds were sown in each of the three plots on May 23, 1956. The mean seed weights (oven dry) of the buckwheat and green grams employed were 30 mg. and 37 mg., respectively. A regular square disposition with a spacing of 10 cm. in both directions was employed in all three plots, such that the density was 100 plants per square metre. In the mixed stand, seeds of each species were sown alternately in both directions, so that any plant of the one species was surrounded by plants of the other species, and 50 plants of each species were found in 1 square metre.

From one or two places selected in each plot, 10 individuals of each species, (therefore 20 from the mixed stand), were sampled at constant intervals of 7 days from June 8 to July 20, with a minimum of disturbance to the remaining plants. The dry weights of the leaves, stems and roots were there measured after stratifying the plant organs into strata of 10 cm. high, in order to reproduce the productive structure of each plant community for illustration of its development. The vertical distribution of the relative light intensity in the community was measured with two electric photometers (Toshiba, No. 5); one of which was placed in the open to measure the full daylight and the other, within the plant community, was used for the measurement of light intensities under the leaf canopy.

The photosynthetic activity was measured at the excised leaf according to the air current method devised by Boysen Jensen [1]. The experiment was car-

ried out at 25°C and in the carbon dioxide tension of the air (0.03 Vol. %). The respiration intensities of the stems and roots were determined by means of the same apparatus at 25°C.

### Results

Table I shows the dry weight of 100 plants of buckwheat and green gram in each plot. It can be seen from the table that in the pure stand, the growth of green grams is somewhat less than that of buckwheat. On July 13, 1956 (52 days after the sowing), the total dry weight of 100 buckwheat plants in the A-1 plot was 329.3 g., while the corresponding figure for the green grams was 265.5 g. The growth of the latter was observed to be noticeably slight in the early stage of development (June 8–June 15), when they had only the first leaves. It appeared that this was chiefly due to the suppression of photosynthesis by the closure of stomata in the daytime.

TABLE 1  
Variation of total dry weight (g.) of 100 plants with time in pure and mixed stands of buckwheat and green gram (A-plots, 1956)

Plot	Plant	June				July		
		8	15	22	29	6	13	20
A-1	Buckwheat	15.0	43.7	121.9	172.1	230.4	329.3	439.5
A-2	Green gram	12.6	14.7	39.8	103.8	129.2	265.5	328.4
A-3	Buckwheat	15.0	43.7	98.1	212.7	311.2	340.7	—
	Green gram	12.6	14.7	24.7	33.9	49.8	67.9	—

In the mixed stand of buckwheat and green grams (A-3 plot), on the other hand, the difference in dry weight between the two species was more striking than in the case of the pure cultures. On July 13, the dry weight of 100 buckwheat plants, 340.7 g., was larger in comparison with the corresponding figure for the green grams of 67.9 g., only a fifth of the 265.5 g. observed in the pure stand on the same day. No reduction in the plant number of the green grams, however, was seen at this stage of growth.

Fig. 1 shows a comparison of the productive structures of 50 buckwheat and green gram plants in pure and mixed stands, which were constructed from the experimental results of July 13, using the stratifying clip method devised by Monsi and Saeki [10]. Although the buckwheats showed a slight lifting of the leaves to the upper position of the community in the pure stand, almost no difference in total plant weight was found between the pure and mixed stands. In the case of the green grams, on the contrary, a marked difference was shown both in total weight of plants and in their structure, between the mixed and pure stands.



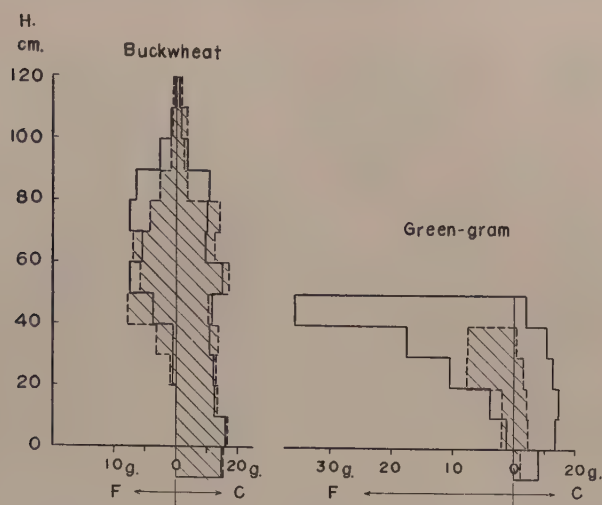


Fig. 1. Comparison of productive structures which were revealed with the stratifying clip method, of 50 buckwheat and green gram plants in pure (solid lines) and mixed stands (broken lines-hatched), on July 13, 1956 (52 days after sowing). Abscissae show dry weights (g.) of photosynthetic system ( $F$ ) and of non-photosynthetic system ( $C$ ) at each 10 cm. thick stratum of the stand.

Calculation of total dry weight of plants per unit area (g./sq. m.) in each of the three plots gave the following results:

	June 22	June 29	July 6	July 13
A-1 (B)	121.9	172.1	230.4	329.3
A-2 (G)	39.8	103.8	129.2	265.5
A-3 (B + G)	61.4	123.3	180.5	204.3

It is obvious from these data that the highest yield of stand was attained in the A-1 plot, while in general the A-2 plot gave the lowest yield, excluding the exceptionally low standing crop of the A-3 (mixed) plot on July 13.

As to the height growth of stem, there exists a significant difference between these two species, as shown in Fig. 2. Buckwheat grew in height at a rate twice as rapid as that of green grams both in the pure and mixed stands.

In order to evaluate the efficiencies of the leaves in the production of matter, the net assimilation rate (NAR, leaf dry weight basis, g./g./week: Watson [12]) was calculated from the experimental results (Table 2). Comparison of NAR between the two species showed that during the early period from June 8 to June 15, the NAR of green grams in the A-1 plot was only 0.26 g./g./week because of the closure of stomata, compared with the value of 2.99 of buckwheat in the A-2 plot, but in the middle and later stages (June 15-July 13) the NAR of green grams reached nearly the same level as that of buckwheat; in

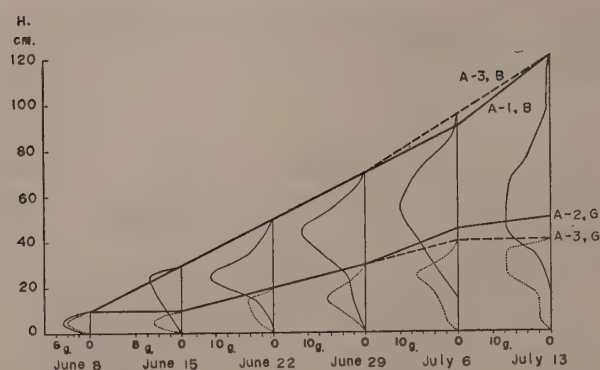


Fig. 2. Changes with time of structure of photosynthetic system and height. Fine solid lines represent the structures of photosynthetic system of buckwheat, and fine dotted lines, those of green grams. Abscissae represent leaf dry weights (g.) of 50 plants at each 10 cm. thick stratum of the stands. Thick solid lines show height growth curve of the two species in the pure stands, and thick broken lines, those in the mixed stand.

TABLE 2

Variation of net assimilation rate (NAR, g./g./week, leaf dry weight basis) with time in pure and mixed stands of buckwheat and green gram (A-plots, 1956)

Plot	Plant	June			July	
		8-15	15-22	22-29	29-6	6-13
A-1	Buckwheat	2.99	3.59	1.09	1.04	1.68
A-2	Green gram	0.26	2.62	2.52	1.24	0.87
A-3	Buckwheat	2.99	2.50	2.40	1.83	0.61
	Green gram	0.26	0.48	0.63	0.80	0.63

one case it even exceeded the NAR of the latter. These results point to the conclusion that the difference in plant weight between the two species in pure stands was chiefly due to the reduced growth of green grams in the earlier stages.

In a previous paper [5], the author has reported that the level of the NAR is chiefly determined by the following characters: the photosynthetic activity of the leaves, the C/F ratio (the ratio of photosynthetic organs to non-photosynthetic organs), and the rate of respiration of the non-photosynthetic organs.

The rate of photosynthesis in the leaves, one of the most important factors affecting on the level of the NAR, was determined under illumination of more than 20 K lux (light saturation). In the case of buckwheat, the maximum rates of photosynthesis were in the range of 5.4-10.8 mg. CO<sub>2</sub>/50 cm.<sup>2</sup>/hr.; while in the case of green grams, the corresponding figures were 8-10.4 mg., showing little difference between them in the maximum rate of photosynthesis. The

$C/F$  ratio, expressed as percentages of the whole plant weight, is presented in Table 3. It is seen that the percentages of the leaves in buckwheat decrease consistently from about 60% to 20% with the development of the plants, while in the case of green grams it was maintained at a constant level of 60-50% throughout the most of the developmental period. As to the rate of respiration of non-photosynthetic organs, there was observed no great difference between the two species. Judging from these results, it is not unreasonable to suggest that the inherent efficiency of production in green grams is greater than, or, at least the same as that in buckwheat, when each species is planted in pure stand.

TABLE 3

Relative proportion of leaves ( $F$ ) and of non-photosynthetic organs ( $C$ ), such as stems, roots and flowers, expressed as percentages of total dry weight of each plant.  
(A-plots, 1956)

Plot	Plant		June				July		
			8	15	22	29	6	13	20
A-1	Buckwheat	$F$	64.0	49.9	37.5	32.4	25.6	21.9	16.7
		$C$	36.0	50.1	62.5	67.6	74.4	78.1	83.3
A-2	Green gram	$F$	63.5	61.8	63.8	58.3	51.8	52.2	48.6
		$C$	36.5	38.2	36.2	41.7	48.2	47.8	51.4
A-3	Buckwheat	$F$	64.0	49.9	41.2	32.6	25.3	21.8	—
		$C$	36.0	50.1	58.8	67.4	74.7	78.2	—
	Green gram	$F$	63.5	61.8	59.5	58.4	57.6	55.7	—
		$C$	36.5	38.2	40.5	41.6	42.4	44.3	—

As has been demonstrated earlier, the growth of green grams was markedly suppressed by the mixed culture with buckwheat; during the period from June 15 to July 6 the values of the NAR for green grams in the mixed stand were  $1/2$ — $1/5$  of those in its pure stand (A-2 plot) (Table 2).

The reason why the growth of green grams was depressed under the condition of mixed culture with buckwheat, can easily be seen from Fig. 2, which shows the vertical distribution of leaves of the two species in the mixed community, determined with the stratifying clip method. As obviously seen from the figure, after June 15 the buckwheat photosynthetic system occupied the upper stratum of the community, seriously shading the green grams. In this situation, the light falling on the green grams must be reduced increasingly with the development of the buckwheat photosynthetic system. The ranges of relative light intensity prevailing on the green grams were as follows: 89-53% of full daylight on June 22, 50-27% on June 29, 35-16% on July 6 and 37-18% on July 13.

The diminution of light intensity must bring about the depression of photosynthesis in the leaves of green grams, and consequently decrease of total



amount of dry matter produced. In order to discuss the quantitative relationship between the light intensity and the productivity of the leaf, the author calculated the daily relative productivity of the green gram leaf in relation to

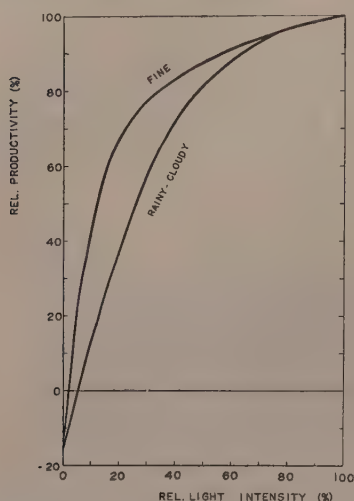


Fig. 3. Relationship between relative light intensity and relative productivity of green gram leaves, on fine and rainy-cloudy days in July.

the relative light intensity received by it (Fig. 3). As the basis of this calculation, the light assimilation curve of the sun leaf of green gram, as illustrated in Fig. 7-1, was used. And as the daily sequence of illumination on fine and rainy-cloudy days, the following mean values determined in July 1955 at Toride, Ibaraki, were employed: at 12hr, 120 K lux and 25 K lux; at 11hr and 13hr, 115 K lux and 25 K lux; at 10hr and 14hr, 80 K lux and 25 K lux; at 9hr and 15hr, 45 K lux and 25 K lux; at 8hr and 16hr, both 25 K lux; at 7hr and 17hr, both 11 K lux; and at 6hr and 18hr, both 2.5 K lux. It can be seen from Fig. 3 that if the light intensity diminishes to 50% or 30% of full daylight, the productivity decreases to 88% or 78% on fine days and to 82% or 58% on rainy-cloudy days, respectively. Such reduction in productivity would accumulate into a significant depression in the total growth of the shaded plants in due course of time. It is, therefore, presumed that the

suppression of the daily productivity through the shading of leaves is chiefly responsible for the decrease in the growth of green grams in the mixed stand.

#### EXPERIMENT B. HETEROCHRONOUS MIXED PLANTING OF BUCKWHEAT AND GREEN GRAMS

In the foregoing section, the author indicated the reduced growth of green grams growing under the leaf canopy of buckwheat in a mixed stand, in which both species were sown on the same day. It may be expected from the results of Experiment A that the growth of green grams in mixed stand will become better if the buckwheat is sown later than the green grams with the result of improvement of light condition for the growth of green grams; and it may even be possible for the green grams to overcome the buckwheat if the latter are planted later enough after the former's appearance. Experiment B was designed to give a quantitative elucidation of this problem from the standpoint of the production of matter by plants.

##### *Methods*

In Experiment B, a series of mixed cultures of buckwheat and green grams was made, beginning May 26, 1956. The preparation of the soil and the methods

of sowing were the same as in the foregoing experiment, except that the two species were sown heterochronously to each other; buckwheat and green grams were sown on the following dates, respectively:

	Green gram	Buckwheat
B-1 plot	May 29	May 26
B-2 plot	May 29	June 4
B-3 plot	May 29	June 11

### Results

The total weight of 100 buckwheat plants and their leaf area index at each stage of development are shown in Fig. 4, in which each value is plotted against the number of days after sowing. Although it can be seen from the results that there was no competitive suppression of growth on the side of buckwheat in any plot of the B-series, there still remains the possibility that the buckwheat might be suppressed if it were planted long after the formation of thick green gram foliage. In fact, it was revealed from another mixed culture experiment that the growth of buckwheat was retarded when it was sown 16 days later than the green gram sowing.

Concerning the growth of green grams, on the contrary, there was seen a significant difference among the three plots in plant weight in the later stages of development. On July 18, 1956 (50 days after sowing) the plant weights in the B-1, B-2 and B-3 plots were in the ratio of 47.6:72.9:100 (cf. Table 4).

The sequences of development of the productive structure by which we can easily understand the vertical distribution of leaves and other organs and of the relative light intensity, are illustrated in Fig. 5 for each of the B plots. Because of the rapider height growth of buckwheat than of green grams, the former were able to extend their photosynthetic systems out over those of the latter in a relatively short time, even in the case when the former were sown 13 days later than the latter. This may be the reason why the growth of buckwheat did not differ among the three plots.

The light condition becomes more unfavourable for the growth of green grams growing under the leaf canopy of buckwheat with the growth of the

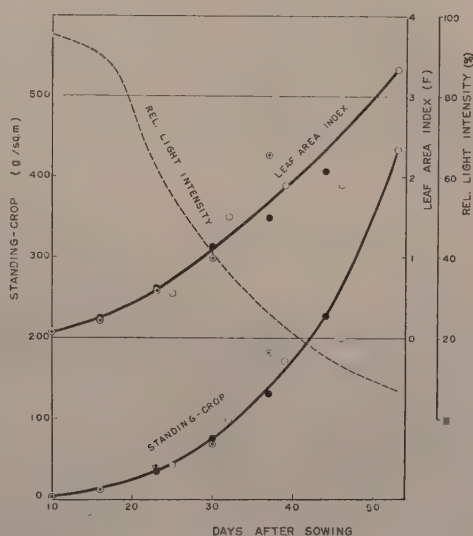


Fig. 4. Changes in total weight of 100 buckwheat plants, their leaf area index and relative light intensity under buckwheat leaf canopy. Values are plotted against number of days after sowing. ○ B-1, ● B-2, ◐ B-3.

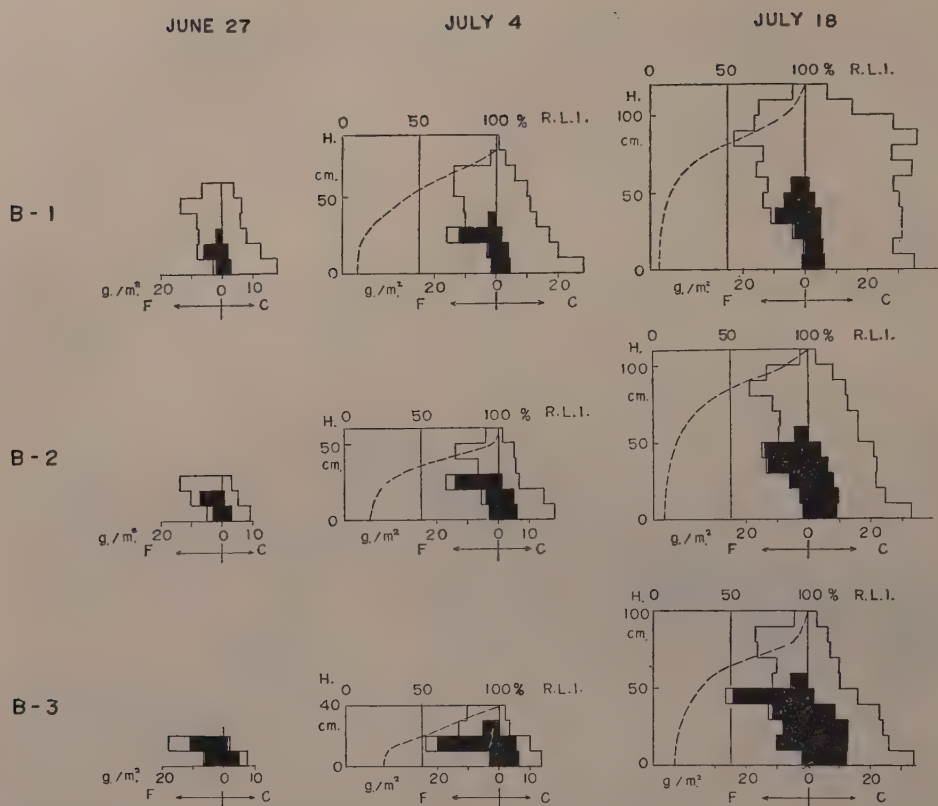


Fig. 5. Sequences of development of productive structure in B plots. Black polygons indicate green grams; open polygons, buckwheat.

Table 4  
Variation with time of total dry weight and total leaf area of 50 plants (B-plots, 1956).  
*Total dry weight (g.) of 50 plants.*

Plot	Plant	Sowing date	June		July		
			20	27	4	11	18
B-1	Buckwheat	May 26	42.5	98.8	171.4	200.2	433.9
	Green gram	May 29	9.2	17.3	32.7	42.2	53.5
B-2	Buckwheat	June 4	12.7	36.0	76.2	131.1	227.9
	Green gram	May 29	11.8	19.2	37.4	50.8	82.0
B-3	Buckwheat	June 11	3.7	12.1	42.4	69.1	182.4
	Green gram	May 29	11.0	25.0	44.6	78.3	112.5



Total leaf area (sq.m.) of 50 plants.

Plot	Plant	Sowing date	June		July		
			20	27	4	11	19
B-1	Buckwheat	May 26	0.55	1.50	1.89	1.90	3.34
	Green gram	May 29	0.17	0.43	0.84	1.42	1.14
B-2	Buckwheat	June 4	0.25	0.62	1.13	1.49	2.07
	Green gram	May 29	0.24	0.39	0.89	1.38	2.06
B-3	Buckwheat	June 11	0.07	0.21	0.79	0.98	2.27
	Green gram	May 29	0.22	0.57	0.85	2.05	2.68

latter. The degree of shading produced was the lowest in the B-3 plot, and the highest in the B-1 plot (Fig. 5). On July 4, for example, the relative light intensity measured at the top layer of green grams was ca. 30% of full daylight in plot B-1 in comparison with ca. 70% in plot B-3. These striking differences in light intensity may be regarded as a main cause of the marked differences in growth of green grams among the three plots.

### THEORETICAL ANALYSIS OF THE GROWTH CURVE OF GREEN GRAMS

In order to elucidate the decisive role of light in the growth of green grams in mixed stands, the author has tried to construct the plant growth curves theoretically, on the basis of computation combining each component acting in the growth process, i.e. photosynthesis, respiration, distribution ratio of dry matter to each organ, and light intensity received by leaves. The curves thus calculated will be compared with the observed ones.

Since the net production ( $P_n$ ) in a plant community is the difference between the total yield of photosynthesis (gross production) and the loss of matter by respiration, it can be expressed by the following formula:

$$P_n = F(a - r) - C \cdot r_o$$

In this formula,  $F$  and  $C$  indicate the amount of leaves and that of non-photosynthetic organs (stems, roots etc.) respectively;  $a$  and  $r$  are the rate of assimilation and respiration of the leaves; and  $r_o$ , the rate of respiration of the non-photosynthetic organs. If we want to calculate the net production of a plant community, the magnitude of these quantities must be determined experimentally throughout the period in question.

#### Gross production ( $F \cdot a$ )

The magnitude of the gross production of plants is proportional to the total leaf area and the rate of assimilation per unit leaf area. The latter depends chiefly on the intensity of light falling on the leaf surface.

To determine the light intensity under which green grams grow, with special reference to the amount of interference from the buckwheat leaves, it was assumed for simplicity of calculation that all the leaves of the latter species were always arranged above those of the former. It can be seen from the productive structures in Fig. 5 that this assumption does not strain the facts much. As mentioned above, the variation with time of the buckwheat leaf area was similar in each of the three mixed stands. As a result the amount of leaf area of buckwheat and, consequently, the relative light intensity under the leaf canopy of buckwheat in the mixed stands can be determined by the number of days after the sowing of buckwheat, assuming that the relationship between light intensity and leaf area is expressed by the following formula (see Monsi and Saeki [10], p. 33):

$$I = I_0 e^{-0.9F},$$

where  $I$  is the light intensity under the leaf canopy,  $I_0$  the incident light intensity, and  $F$  the leaf area index. With giving the value for buckwheat to this formula, we can readily determine the original light intensity reached at the uppermost surface of the green gram leaves, and its trend toward decreasing with lapse of days after sowing is shown in Fig. 4 (broken line). The intensity of light which is received by green gram leaves assimilating in their own shade was also calculated by the same formula.

In a previous paper [5] discussing the density effect of buckwheat, the gross production was calculated from a single light-assimilation curve, assuming that the rate of photosynthesis of old leaves is the same as that of new ones. In the case of green grams, however, a marked decline in photosynthesis with

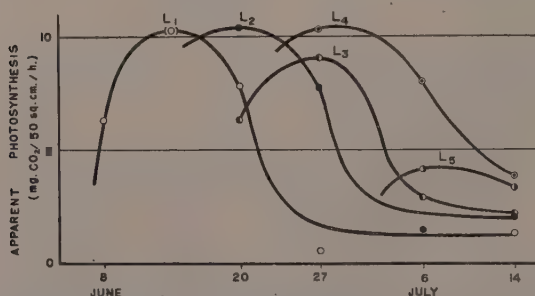


Fig. 6. Change with time of maximum rate of photosynthesis in green gram leaves.

lapse of time was observed, and the continuation of the maximum rate (ca. 10 mg.  $\text{CO}_2$  / 50 sq. cm./hr.) was only about 1 week, as shown in Fig. 6. For the sake of the theoretical calculation of dry matter production by green grams, therefore, it was assumed that the maximum rate of photosynthesis at 20 K lux is 10.3 mg.  $\text{CO}_2$  / 50 sq. cm./hr. for leaves which were in the first week after their emergence; 6.0 mg. for those in the second week, and 2.0 mg. for those in the third week. Using these assumptions, the author composed three light-assimilation curves, as demonstrated in Fig. 7. Again daily photosynthesis curves related to the relative light intensity should be prepared for further calculation of gross production of the green grams. The curves of Fig. 8 were composed on the basis of the above-mentioned light-assimilation curves in the three stages of leaf maturity and the measurement of daily marches of illumi-

nation on fine, light-cloudy and rainy-cloudy days at Toride in July 1955 [5]. Other procedures of the calculation were in general the same as those in the previous paper.

Fig. 7. Light assimilation curves of green grams, which were used for the calculation. 1, 2 and 3 indicate the curves of leaves which are in the first, second and third week after their emergence.

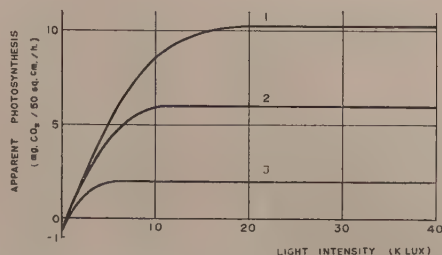
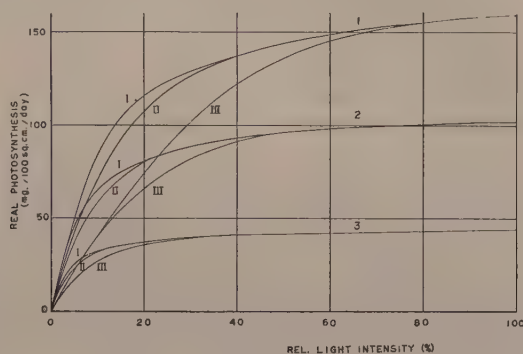


Fig. 8. Relationship between photosynthesis by green gram and relative light intensity. I, II and III show the curves on fine, light-cloudy and rainy-cloudy days, respectively.



### *Dry matter loss by respiration*

The determination of respiration rate in each organ, carried out at 25°C, is shown in the following:  $\text{CO}_2$  output of leaves was 0.78 mg./50 sq. cm./hr. and those of stems and roots were in the range of 2-5 mg. and 6-7 mg./g.D.W./hr., respectively. From these measurements mean values were calculated for the purpose of determination of dry matter loss by respiration. All the values were expressed as the daily loss of carbohydrate per unit amount of each organ, on the assumption that 1 g. of  $\text{CO}_2$  is equivalent to 0.61 g. carbohydrate. For leaves the value of 0.023 g./100 sq. cm./day was employed; for stems and roots, 0.057 and 0.098 g./g. D.W./day, respectively.

### *Distribution of the dry matter produced*

The difference between the gross production and the loss by respiration is the net production ( $P_n$ ), which will be distributed over various organs and utilized for development of new leaves, stems, roots and reproductive organs. In Table 5-b are brought out the distribution ratios of the dry matter increment of green grams, calculated from the experimental data. With the values of the table and the amount of  $P_n$ , there is no difficulty in estimating the amount of new leaves ( $\Delta F$ ), and that of new shoots and roots in the non-photosynthetic systems ( $\Delta C$ ), for each successive stage of development.



TABLE 5a

Relative proportion of leaves, stems and roots, expressed as percentage of total plant weight of green gram (B-plots, 1956)

Plot		June		July		
		20	27	4	11	18
B-1	Leaves	59.8	57.2	55.4	51.9	49.5
	Stems	27.2	29.5	35.1	40.8	44.5
	Roots	13.0	13.3	9.5	7.3	6.0
B-2	Leaves	65.3	61.5	57.5	55.3	51.7
	Stems	24.1	28.2	32.0	37.7	43.7
	Roots	10.6	10.3	10.5	7.0	4.6
B-3	Leaves	64.6	68.0	63.8	57.8	53.3
	Stems	25.4	24.4	28.5	35.6	42.1
	Roots	10.0	7.6	7.7	6.6	4.6

TABLE 5b

Distribution ratio of dry matter increment to individual organs of green gram (B-plots, 1956)

Plot		June 20-27	June July 27-4	July 4-11	July 11-18
B-1	Leaves	54.4%	53.1%	41.5%	40.7%
	Stems	32.1	42.0	57.5	58.4
	Roots	13.5	4.9	1.0	0.9
B-2	Leaves	55.4	51.3	51.9	45.9
	Stems	34.5	38.0	47.1	53.1
	Roots	10.1	10.7	1.0	1.0
B-3	Leaves	70.7	58.7	49.4	43.2
	Stems	23.6	33.5	45.7	56.8
	Roots	5.7	7.8	4.9	0.0

#### *Relation between leaf weight and leaf area*

Since the photosynthetic activity of leaves is usually measured on a leaf area basis, and the interception of light is related to total area of leaves, the increment of leaves ( $\Delta F$ ) expressed as dry weight must be converted into leaf area. The conversion was done on the basis of measurement of the relationship between leaf area and leaf dry weight as in Table 6. It is obvious from the table that the value was the highest in the B-1 plot and the lowest in the B-3 plot throughout most of the developmental stages; on July 4 the value was 459 sq. cm./g. D.W. in the B-1 plot, while in the B-3 plot it was 292 sq. cm./g. D.W. Green grams in the B-1 plot were, therefore, able to produce a leaf area about one and half times as large as in the B-3 plot from the same amount

TABLE 6

Leaf area (sq. cm.) per 1 g. dry weight of leaves of green gram in mixed stand with buckwheat (B-plots, 1956)

Plot	June		July		
	20	27	4	11	18
B-1	309	434	459	645	430
B-2	305	366	425	495	486
B-3	313	334	292	455	447

TABLE 7

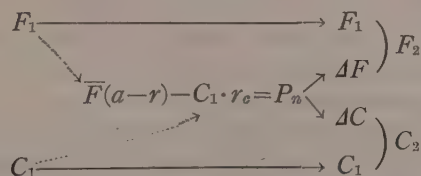
Leaf area (sq. cm.) per 1 g. leaf dry weight of green gram grown under various light intensities (100, 75, 50, 30 and 20% of full daylight). Sown on August 6, 1955.

Light intensity	100%	75%	50%	30%	20%
August 15	382	479	600	685	637
August 23	558	710	879	1211	1340

of dry matter.

A similar result was observed in pot culture of green grams under various light intensities (100-20%). The result of this pot experiment, which was carried out in August 1955, has shown that the area of the leaf increased with the decrease in the relative light intensity (Table 7). Although green grams may be favoured by this character to a great extent in the production of matter under reduced light, this sort of advantage is cancelled by the serious depression of net assimilation under weakened light and by the reduction of dry matter distributed to the leaves.

The whole process of the production and reproduction of dry matter in annual plants can be summed up in the following schema, which was introduced by Monsi (unpublished):



where  $F_1$  and  $F_2$  represent the weight of the leaves,  $\overline{F}$  the leaf area,  $C_1$  and  $C_2$  the weight of the non-photosynthetic organs. The meanings of  $a$ ,  $r$ ,  $r_e$  and  $P_n$  have been defined above. By giving the defined values to all the quantities of this schema, we are able to calculate the production and the distribution of dry matter for each stage, and thus to estimate the dry weight of the plants after

a definite period of time.

The calculation was carried out for the period from June 20 to July 18. As the starting point of this calculation, the mean values of dry weights for leaves, stems and roots obtained at the three plots on June 20 were employed; the

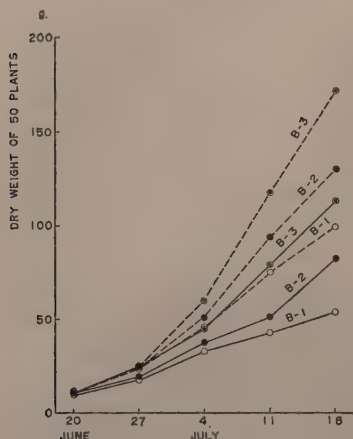


Fig. 9. Growth curves of 50 green gram plants. Broken lines show the theoretical values.

mean dry weight per 1 square metre of land area was 6.6 g. for leaves (leaf area=20.3 sq. dm.), 2.7 g. for stems and 1.2 g. for roots. The theoretical growth curves thus obtained are shown with the broken line in Fig. 9.

The trends of the theoretical growth curves thus constructed have demonstrated marked differences among the three plots; the calculated plant weights in the B-3, B-2 and B-1 plots were in the ratio of 100:76.0:58.0, on July 18. This ratio accords fairly well with the corresponding ratio of 100:72.9:47.7, which was directly observed on the same day, although the absolute values calculated were nearly twice as large as those of the direct measurement. With regard to the reason why the calculated values became larger than the observed, some discussion will be given in the following section.

It may be concluded from the above results that the differences in the growth rate of green grams among the three plots were chiefly caused by the differences in the productive structure of these mixed plant communities and particularly in the light intensity at the level of green grams, and that the height growth of plants plays an important role in determining the fate of interspecific competition in the plant community.

## DISCUSSION

The experimental results presented above have suggested that in mixed vegetation the competitive ability of a plant species can not be directly determined only by the rate of its growth in weight or by its efficiency in dry matter production as measured in a pure state of culture of a single species, and that it is possible, in an extreme case, that a species of higher productivity will lose in competition with other species of lower productivity. In the present experiment green grams (A-plots), showing in the pure stand a growth rate similar to that of buckwheat, were defeated in competition with the latter, with the result of a marked suppression of growth in the green grams through the shading effect of the buckwheat leaves.

Some investigators such as Walter [11] and Kramer and Decker [6] have already defined the photosynthetic activity, or the ratio of non-photosynthetic to photosynthetic system, etc., as the important factors which determine the result



of competition among plant species. Such a direction of research is no doubt imperative in elucidating the problem of plant competition on a quantitative basis. But besides this we must consider the role of height growth (the elongation of stems or boles) in nature, because of its significance in intraspecific competition. Plants with higher rates of height growth may develop their photosynthetic systems in the upper part of the plant community and thus may suppress the growth of the other plants.

For more rapid growth in height, plants must be provided with a greater amount of dry matter for stem growth. In many cases, a plant species with a higher rate of height growth distributes for stem growth a larger proportion of the total increment of dry matter than one with a lower rate. The same is also true in the relation between buckwheat and green grams. The former's distribution ratio to the stems was considerably higher than that of green grams (Table 3-b). It is already made clear in a previous paper [5] that the productivity of plants in the pure culture becomes higher as the distribution ratio of dry matter to leaves increases. The case is, however, different in a mixed plant community. The results of the present experiment have demonstrated that the larger distribution ratio to stems of plants may, within a certain limit, cause a higher efficiency of the plants in dry matter production through the improved light condition and, consequently, give them a more effective weapon in the competition with other plant species.

In fields where the competition between crop plants and weeds is allowed to follow its natural course, we may often observe the growth of the crops being suppressed by the weeds which grow more luxuriantly. This suppression of growth may be presumed to be brought about through the following mechanism. In crop plants the distribution ratio of dry matter to leaves is in general so high as to enable the plant to reach its maximum of leaf area index in a relatively short period. This is certainly one of the important characteristics of crops which are required to possess higher productivity or more rapid growth (cf. Watson, [12]), and which are usually planted under man's control without any competition with other species. In the case of the weeds, on the contrary, the distribution ratio to stems is often higher than that of the crop plant. This characteristic will secure the more rapid growth in height of the weed. It is presumed that the weed is able to shade the crops through its rapid growth of stem and thus reduce the growth of the crop plant.

In the previous section the author has calculated the standing crops of green grams in the B-plots to compare the calculated values with the observed, and found that the estimated values of July 18 (50 days after the sowing) were about one and a half to two times as large as the values directly measured. The reasons for this seem to be the following:

- 1) The calculation of the gross production was carried out on the basis of the light assimilation curve of a leaf with wide-open stomata. In the earlier developmental stages of the green grams, however, such marked closure of stomata took place as to result in serious depression of the growth. Therefore it may be thought that the divergence of the estimated values from the direct

measurements was chiefly caused by the closure of the stomata in the earlier stages.

2) The leaves of green grams in the mixed stands were observed to be converted into the shade-leaf type; their thickness being more or less reduced as the plants develop, as the result of shading by buckwheat leaves. In the B-1 plot, for instance, leaf area per 1 g. leaf weight (oven dry) of June 20 was 309 sq. cm. compared with 645 sq. cm. of July 18. Thus in the later stages (July 18) more than twice the leaf area in the earlier stages was built up from the same amount of dry matter distributed to the leaves. The gross production was calculated in the present work on the assumption that the photosynthetic activity per unit leaf area was the same, regardless of the thickness of the leaves. In the mixed stand of green grams and buckwheat, however, photosynthetic activity of green gram leaves was observed in the later stages by Saeki (unpublished) to become the shade leaf type with a reduced rate of maximum assimilation. The maximum rate of assimilation in the third leaves grown under the reduced light intensity was measured to be only 5 mg.  $\text{CO}_2$ /50 sq. cm./hr. in comparison with the corresponding figures of 11-12 mg.  $\text{CO}_2$  in the first and second leaves which were grown under relatively good light conditions. This suggests that the photosynthetic ability may have declined as the leaf thickness decreases. So it is likely that the level of photosynthetic activity on which the calculation of production by green grams was based may have been overestimated. This seems to be a major reason why the estimated values exceeded the observed. It may be expected, moreover, that the calculated values of the standing crop will approach considerably closer to the observed ones if the calculation is performed on the basis of the photosynthetic activity, expressed on a leaf weight basis rather than a leaf area basis.

## SUMMARY

The interspecific competition in a plant community was investigated experimentally with synchronous (A-plots) and heterochronous (B-plots) mixed stands of buckwheat (*Fagopyrum esculentum*) and green grams (*Phaseolus viridissimus*), on the basis of the dry matter production of the constituent species.

1) Growth rates in weight of buckwheat and green grams in pure stands (A-1, A-2) were noticeably similar. But in the mixed stand (A-3) there was seen marked suppression of the growth of green gram, especially of the leaves, by the buckwheat (Table 1 and 2).

2) In another series of experiments, seeds of the buckwheat were sown in heterochronous mixtures 3 days before (B-1 plot), 6 days after (B-2 plot), and 13 days after (B-3 plot), the sowing of green grams. Because of its faster growth in height, buckwheat was not suppressed by green grams, even in the B-3 plot. An increase in dry weight and leaf area of buckwheat was almost similar in the three plots (Fig. 4). In the case of green grams, however, there was marked difference in growth rate among the three plots. On July 18, 1956, at the end of the experiment, the ratio of dry weight was 47.6:72.9:100 (Table 4 and

Fig. 9).

3) The maximum rate of photosynthesis of green grams was about the same as buckwheat, i.e. 10–11 mg.  $\text{CO}_2$ /50 sq. cm./hr. However, the photosynthetic activity of a leaf of green grams could remain in the maximum only for about one week after maturing of the leaf, and thereafter it declined and soon reached a final activity of 2 cm.  $\text{CO}_2$ /50 sq. cm./hr. (Fig. 6).

4) The relative proportion of the leaves expressed as the percentage of the total plant weight (oven dry), was larger in green grams (60–50%) than in buckwheat (60–20%) (Table 3).

5) During the period from June 15 to July 13, there was not much difference in the net assimilation rate (NAR) between buckwheat (A-1, 3.59–1.04 g./g. dry weight/week) and green gram (A-2, 2.62–0.87 g./g./week) which were planted in pure stands. However, in the mixed stand (A-3), the NAR of buckwheat (2.50–0.61 g./g./week) was markedly higher than that of green gram (0.80–0.48 g./g./week) (Table 2).

6) The first of the important factors which affect the final result of the competition between buckwheat and green grams seems to be the height growth of these plants. Buckwheat showed a growth rate in stem elongation twice as rapid as that found in green grams, and were thus able to shade the latter. With the development of the buckwheat foliage, the light intensity received by the suppressed green grams was diminished to 35–16% of full daylight. Therefore, productivity of green grams with such weak illumination should become very low (Fig. 3).

7) The growth curves of green gram plants in the three B plots were constructed theoretically, on the basis of photosynthesis (Figs. 7 and 8), respiration, distribution ratio of dry matter increment to each organ, such as leaves, stems and roots (Table 5b), and the light intensities received by the leaves. The growth curves thus constructed were compared with those directly measured, showing a marked difference in growth rate among the three plots similar to that found in the direct measurement (Fig. 9).

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# CYTOGENETICAL STUDIES ON THE INTERGENERIC F<sub>1</sub> HYBRIDS BETWEEN *TRITICUM MACHA* AND FOUR SPECIES OF *SECALE*

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## I. INTRODUCTION

The hybridization between Dinkel wheat and *Secale* was made at first with *vulgare*, and the achievements of its cytological and genetical researches have been reported by many authors, such as Kihara (1919, 1924), Meister and Meister (1924), Thompson (1926) and others. Then the hybridization was done also with *compactum*, *Spelta* and *sphaerococcum* to *Secale*, by such researchers as Kagawa and Chizaki [2] and Nakajima [6, 7, 8, 9]. And its cytological and genetical studies were made in these hybrids.

However, no report seems to have been published on the cytological and genetical research in the hybrid raised from *Macha* and *Secale*.

As one of a series of cytogenetical studies on intergeneric hybrids between *Triticum* and *Secale*, the present author tried to make out four hybrids between *T. Macha* and each of 4 species of *Secale* (*cereale*, *Vavilovii*, *africanum* and *montanum*) in 1952~'53, and obtained several F<sub>1</sub> plants from the four combinations. Some cytogenetical researches on these F<sub>1</sub> plants were made and the results obtained will be dealt with in this paper.

## II. MATERIAL AND METHODS

*Triticum Macha* used as the mother plant was sent from Kyoto University (Faculty of Agriculture). The seed of *Secale africanum* and *montanum* employed as the pollen parent, was sent from Prof. Arne Müntzing of Lund University, and that of *S. Vavilovii* also used as pollen parent was given by Dr. T. Kawatani. And as to *S. cereale*, the strain cultivated by the present author since 1928 was employed.

The root tip cells and anthers fixed with either Nawashin's or Carnoy's fluid, were used for the cytological study of somatic chromosomes and meiosis was studied in the same method as described in the previous papers by the present author on wheat-rye hybrids. Original magnification of figures is 3000× for the somatic chromosomes and 2300× for the meiotic chromosomes.

## III. RESULTS OF HYBRIDIZATION

The intergeneric hybridization between *T. Macha* and each of four species of *Secale* (*cereale*, *Vavilovii*, *africanum* and *montanum*) was carried out in 1952~

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TABLE 1

The results of hybridization between *T. Macha* and each of four species of *Secale*

Combination of hybridization	Number of spikes	Number of flowers	Number of kernels	Percentage of seed setting
<i>T. Macha</i> × <i>S. cereale</i>	75	1387	37	2.68
" × <i>S. Vavilovii</i>	49	947	52	5.49
" × <i>S. africanum</i>	40	719	16	2.23
" × <i>S. montanum</i>	126	1566	145	9.27

1953, and the results of the hybridization were as shown in Table 1.

As shown in Table 1 the seed fertility in the hybrid between *T. Macha* and *S. montanum* is the highest; that of the hybrid with *Vavilovii* comes next; and that of the hybrid with *africanum* is the lowest. The diversity of percentage may depend on the nature of the *Secale* species employed.

According to the present author [7, 9], the percentage of seed setting in the  $F_1$  plants between *T. vulgare* and *Secale* were 24.86~75.44%, while Totsu (1950) reported it to be 2.1~66.3%.

Thus the percentage of seed fertility of the intergeneric hybrid differs exceedingly in different species of *Triticum* and *Secale* used. The seed obtained by this hybridization was sown in October of the same year and many  $F_1$  plants were obtained. The percentage of  $F_1$  plants raised to the number of seed sown is shown in Table 2.

TABLE 2

Germination of  $F_1$  seeds

Combination of hybridization	Number of seeds sown	Number of germinated seeds	Percentage of germination	Number of plants died in winter	Number of matured plants	Percentage of $F_1$ to pollinated flowers
<i>T. Macha</i> × <i>S. cereale</i>	37	37	100.00	9	28	2.02
" × <i>S. Vavilovii</i>	52	40	76.92	2	38	4.01
" × <i>S. africanum</i>	16	15	93.75	6	9	1.25
" × <i>S. montanum</i>	80	67	83.75	6	61	7.76

As seen in Table 2, the proportion of the  $F_1$  plants to the pollinated flowers shows the highest percentage in the hybrid with *montanum* as that of the seed fertility, next comes that of the  $F_1$  with *Vavilovii*, and that of the  $F_1$  with *africanum* is the lowest. Thus the diversity of percentage may depend on the nature of pollen parents used, as was mentioned above.

According to the present author [9], the percentage of the  $F_1$  plants to the pollinated flowers in the hybridization between *T. vulgare* and 3 species of *Secale* (*Vavilovii*, *africanum*, *montanum*) ranged from 10.74 to 41.32 percent, and that of the hybridization with *cereale* was 25.18 percent [7]. And these values show



remarkably higher than that of the present hybridization between *Macha* and four species of *Secale*. In this case, the diversity of the seed setting percentage of the hybridization is due to the nature of the mother plant used.

The four hybrids raised by the hybridization between the plants mentioned above will hereafter be represented by the following symbols: *T. Macha* × *S. cereale* = MachScF<sub>1</sub>, *T. Macha* × *S. Vavilovii* = MachSVF<sub>1</sub>, *T. Macha* × *S. africanum* = MachSaF<sub>1</sub> and *T. Macha* × *S. montanum* = MachSmF<sub>1</sub>.

#### IV. EXTERNAL CHARACTERISTICS OF F<sub>1</sub> PLANTS

As shown in Table 2, the number of the F<sub>1</sub> plants obtained was 28 for MachScF<sub>1</sub>, 38 for MachSVF<sub>1</sub>, 9 for MachSaF<sub>1</sub> and 61 for MachSmF<sub>1</sub>. Some individual differences, though not remarkable, were observed in the external characteristics of these F<sub>1</sub> plants as shown in Table 3.

TABLE 3  
External characteristics of F<sub>1</sub> plants and their parental plants

Plants	Characters	Number of culms and spikes measured	Average length of culms cm	Average length of spikes cm	Average length of awns cm	Number of spikelets per spike	Spike density	Number of flowers per spikelet	Number of tiller-ings
<i>T. Macha</i>		20	118.30	11.55	3.75	23.25	2.01	3	111.50
<i>S. cereale</i>		20	132.35	13.08	2.75	44.70	3.42	2	115.30
<i>S. Vavilovii</i>		25	141.90	15.44	0.92	47.20	3.06	2	100.00
<i>S. africanum</i>		10	130.41	13.60	0.00	50.40	3.71	2	124.00
<i>S. montanum</i>		20	110.00	15.23	1.23	44.60	2.93	2	
MachScF <sub>1</sub>		90	117.00	15.24	2.35	34.87	2.29	3	100.11
MachSVF <sub>1</sub>		90	119.66	17.42	4.34	34.79	2.00	3	121.56
MachSaF <sub>1</sub>		60	133.17	14.82	2.53	34.72	2.35	3	211.33
MachSmF <sub>1</sub>		150	128.04	15.49	3.47	33.65	2.18	3	115.33

In Table 3 and Photos 1-4, the external characteristics of F<sub>1</sub> plants are compared with those of the parental plants. The culm height of MachScF<sub>1</sub> and MachSVF<sub>1</sub> is less than that of both parents, while the culm height of MachSaF<sub>1</sub> and MachSmF<sub>1</sub> is rather more than that of both parents. In the characteristics of spikes, the number of spikelets per spike and the spike density, were almost intermediate between the parents in every combination, and the lengths of spikes and awns of F<sub>1</sub> were superior to that of both parents in every combination excepting MachScF<sub>1</sub> which was inferior to that of both parents in lengths of spikes and awns. And the number of flowers per spikelet of the F<sub>1</sub> is the same as that of the mother plant.

Generally speaking, though the F<sub>1</sub> plants possess external characteristics of both parents, they resemble more closely the mother plant, *T. Macha*, than to be intermediate. In every F<sub>1</sub> plant, the neck of the spike has no hair is a



Photos. 1-4. Spikes of  $F_1$  and its parents, from left to right: mother plant,  $F_1$  and pollen plant.  $\times ca \frac{1}{2} \times \frac{3}{4}$ .

1. *T. Macha*, MachSc $F_1$  and *S. cereale*. 2. *T. Macha*, MachSV $F_1$  and *S. Vavilovii*.
3. *T. Macha*, MachSa $F_1$  and *S. africanum*. 4. *T. Macha*, MachSm $F_1$  and *S. montanum*.

common characteristic of  $F_1$  plants between *Triticum-Secale*.

The spikelets of MachSc $F_1$  and MachSV $F_1$  were not brittle at the ripening stage, while those of MachSa $F_1$  and MachSm $F_1$  were brittle. This characteristic may have been brought from the natural characteristics of both *S. africanum* and *S. montanum* which were used as pollen plants.

According to the present author [9, 10, 11], the spikelets of  $F_1$  plants between *T. vulgare*, *T. persicum*, *T. turgidum* and *T. durum* and either *S. africanum* or *S. montanum* were brittle at the ripening stage, while those of the  $F_1$  between *T. compactum* and either of *S. africanum* and *S. montanum* were not brittle like the  $F_1$  between many species of *Triticum* and either of *S. cereale* and *S. Vavilovii*, though there were some brittle  $F_1$  plants as an exception, for instance, the combination of *T. dicoccum*  $\times$  *S. cereale*.

## V. SEED FERTILITY OF $F_1$ PLANTS

Every  $F_1$  hybrid in the present researches shows fertility in natural selfing though not in a high percentage. The results are shown in Table 4.

As is evident from Table 4, the seed fertility of MachSc $F_1$  showed the

TABLE 4  
Seed fertility of  $F_1$  plants

$F_1$	Number of individuals	Number of spikes	Number of spikelets	Number of kernels	Percentage of seed setting per spikelet
MachSc $F_1$	9	90	3138	92	2.93
MachSV $F_1$	9	90	3131	1	0.03
MachSa $F_1$	6	60	2083	23	1.10
MachSm $F_1$	15	150	5048	20	0.40

highest, and next comes that of MachSa $F_1$ , that of MachSV $F_1$  being the lowest. The differences of seed fertility may be said to have taken place by the different influences caused by the different *Secale* used as the pollen parent though the mother plant is common. In general, the value of seed fertility was considerably lower than that of the  $F_1$  hybrid between *T. vulgare* and *T. compactum* and each of 4 species of *Secale* (*cereale*, *Vavilovii*, *africanum* and *montanum*) [6, 9, 10, 11], and this result may have been caused by the nature of *T. Macha*.

#### VI. CHROMOSOME NUMBER OF $F_1$ PLANTS

The somatic number of chromosomes of the  $F_1$  plants obtained from the 4 combinations mentioned above, was 28 in every individual examined of each combination (Figs. 1-4). This number corresponds to the sum of the gametic number of the parents, viz.,  $21+7=28$ . In the meiosis of PMC's the same number of chromosomes was also observed as in the root tip cells.

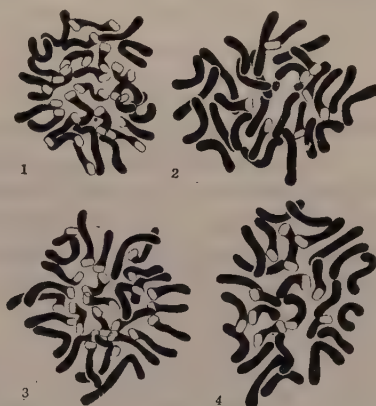
#### VII. MATURATION DIVISION IN PMC'S

##### 1. MachSc $F_1$ plants

Among the 9 individuals, 8 have the number of bivalents 0~4 in PMC's at heterotypic metaphase, and the remaining one has 0~3. The frequency of bivalents in PMC's of the  $F_1$  is as shown in Table 5.

As seen in Table 5, the mode of occurrence of bivalents per plant, was found to have the value 2 in 2 plants, one in 6 plants and 0 for the remaining one.

Throughout the whole 9 plants the configuration that has one bivalent appeared to be the mode.



Figs. 1-4. Somatic plates in root tip cells of  $F_1$  plants between *T. Macha* and 4 species of *Secale*.  $\times 1200$ .

1. MachSc $F_1$   $2n=28$ .
2. MachSV $F_1$   $2n=28$ .
3. MachSa $F_1$   $2n=28$ .
4. MachSm $F_1$   $2n=28$ .



TABLE 5  
Frequency of bivalents at IM of PMC's of MachScF<sub>1</sub> plants

Individuals	Number of bivalents						Total
	0	1	2	3	4	Mode (%)	
MachScF <sub>1</sub> -1	153	197	132	56	12	1 <sub>II</sub> (35.82)	550
" 2	116	194	157	45	18	1 <sub>II</sub> (36.60)	530
" 3	194	183	119	27	7	0 <sub>II</sub> (36.60)	530
" 5	147	213	140	59	11	1 <sub>II</sub> (37.37)	570
" 6	179	192	204	77	18	2 <sub>II</sub> (30.45)	670
" 8	128	313	97	22		1 <sub>II</sub> (55.89)	560
" 9	144	181	175	46	14	1 <sub>II</sub> (32.32)	560
" 10	164	224	234	54	4	2 <sub>II</sub> (34.41)	680
" 12	176	249	162	56	7	1 <sub>II</sub> (44.46)	650
Total	1401	1949	1420	442	91	1 <sub>II</sub>	5300
%	26.43	36.72	26.79	8.34	1.71	36.72	100.00

Trivalents were observed in 2 per 1000 PMC's besides bivalents at the heterotypic metaphase, but no tetravalent was found. These were V-shaped.

Normally, all univalents are scattered in the spindle at the metaphase of heterotypic division as in *Triticum-Secale* F<sub>1</sub>, but in this case the equatorial plate consisting of all the univalents was observed occasionally in every individual case, and all the univalents showed longitudinal splitting. Half of them distributed to the poles, consequently the pollen that has amphidiploid number

TABLE 6  
Occurrence of the F<sub>1</sub>-type division and the formation of equatorial plate at the heterotypic division in PMC's of MachScF<sub>1</sub> plants

Individuals	F <sub>1</sub> -type division		Formation of equatorial plate		Total (%)
	Number	%	Number	%	
MachScF <sub>1</sub> -1	327	34.49	650	65.51	977 (100.00)
" 2	2443	98.55	36	1.45	2479 (100.00)
" 3	816	90.57	85	9.43	901 (100.00)
" 5	1099	98.04	22	1.96	1121 (100.00)
" 6	707	97.38	19	2.62	726 (100.00)
" 8	949	91.16	92	8.84	1041 (100.00)
" 9	421	100.00	0	0.00	421 (100.00)
" 10	694	97.75	16	2.25	710 (100.00)
" 12	1064	82.55	225	7.45	1289 (100.00)
Total	8520	88.15	1145	11.85	9665 (100.00)

of chromosomes may be produced by this process. They play an important role in the formation of amphidiploids. The percentage of the formation of the plate for total number of PMC's is shown in Table 6.

As shown in Table 6, PMC's that show the  $F_1$ -type division mixing with those that have univalents on the equatorial plate, were occasionally observed, but sometimes PMC's showing the  $F_1$ -type division alone were observed.

To sum up, the formation of the equatorial plate is considerably less (11.85%) as compared with the  $F_1$ -type division (88.15%).

In  $F_1$ -type division, the distribution of chromosomes to opposite poles at the ana-telophase in heterotypic division proceeded at random, viz., cases of 14:14~28:0 were observed, but in most cases, the number of chromosomes distributed to the poles was usually not so different for both poles.

Some individuals of this hybrid were fertile by natural selfing, the average value being 2.93% (Table 4).

## 2. *MachSVF<sub>1</sub>* plants

Among the 38  $F_1$  plants, 6 individuals were taken for cytological research. The number of bivalents found in the one PMC at heterotypic metaphase was 0~4 in 2 individuals and 0~5 in one. The frequency of bivalents in one PMC is shown in the following Table 7.

TABLE 7  
Frequency of bivalents at IM of PMC's of *MachSVF<sub>1</sub>* plants

Individuals	Number of bivalents							Total
	0	1	2	3	4	5	Mode (%)	
<i>MachSVF<sub>1</sub></i> -1	186	193	84	26	9	2	1 <sub>II</sub> (38.60)	500
" 4	168	249	63	17	2	1	1 <sub>II</sub> (49.80)	500
" 8	136	197	123	34	8	2	1 <sub>II</sub> (39.40)	500
" 12	131	149	130	58	25	7	1 <sub>II</sub> (29.80)	500
" 13	197	248	45	9	1		1 <sub>II</sub> (49.60)	500
" 15	152	206	106	30	6		1 <sub>II</sub> (41.20)	500
Total	970	1242	551	174	51	12	1 <sub>II</sub>	3000
%	32.33	41.40	18.37	5.80	1.70	0.40	41.40	100.00

The configuration showing one bivalent appeared to be the mode for each of 6 individuals.

A V-shaped trivalent was rarely observed (3 in 1000 PMC's); in a PMC there were bivalents at heterotypic metaphase, but no tetravalent was found.

The distribution of chromosomes to the opposite poles at heterotypic metaphase proceeded at random. In most cases, the distribution was usually not so different for both poles, but at times a very unbalanced distribution of chromosomes at the poles was observed. The frequency of chromosome groups of distribution is shown in Table 8.



Figs. 5-11. Meiosis in PMC's of MachScF<sub>1</sub> hybrid, heterotypic division.  $\times 770$ .

5. 28 univalents scattered in spindle (MachScF<sub>1</sub>-1). 6. Metaphase, side view,  $1_{II}+26_I$  (MachScF<sub>1</sub>-3). 7. do.  $2_{II}+24_I$  (MachScF<sub>1</sub>-5). 8. do.  $3_{II}+22_I$  (MachScF<sub>1</sub>-9). 9. do.  $4_{II}+20_I$  (MachScF<sub>1</sub>-1). 10. Equatorial plate consisted of all univalents, side view (MachScF<sub>1</sub>-1). 11. Metaphase, side view, giant cell,  $1_{II}+26_I$  (MachScF<sub>1</sub>-9).  $\times 570$ .

Figs. 12-17. Meiosis in PMC's of MachSVF<sub>1</sub> hybrid, heterotypic division.  $\times 770$ .

12. 28 univalents scattered in spindle (MachSVF<sub>1</sub>-8). 13. Metaphase, side view,  $1_{II}+26_I$  (MachSVF<sub>1</sub>-1). 14. do.  $2_{II}+24_I$  (MachSVF<sub>1</sub>-1). 15. do.  $3_{II}+22_I$  (MachSVF<sub>1</sub>-1). 16. do.  $4_{II}+20_I$  (MachSVF<sub>1</sub>-8). 17. do.  $5_{II}+18_I$  (MachSVF<sub>1</sub>-12).

The cells of the tetrad stage consisting of 2~6 cells were observed, and the case of those consisting of 4 cells appeared to be the mode.

### 3. MachSaF<sub>1</sub> plants

In this combination, 9 F<sub>1</sub> individuals were obtained, but 6 plants were used



TABLE 8  
Distribution of chromosomes to the poles at the heterotypic  
anaphase in PMC's of MachSVF<sub>1</sub> plants

Individuals	Distribution of chromosomes											Total
	5:23	6:22	7:21	8:20	9:19	10:18	11:17	12:16	13:15	14:14	Mode	
MachSVF <sub>1</sub> -1	2		3	4	3		2	4	6	5	13:15	29
" 4		1			1	3	4	4	5	8	14:14	26
" 8		1	1	2	2	6	3	7	3	6	12:16	31
" 12	2	2	1	2	1	1	1	2	2	5	14:14	19
" 13	3	2	1	3	2	1	2	5	9	11	14:14	39
" 15		1	2	1	1	2	1	3	3	5	14:14	19
Total	7	7	8	12	10	13	13	25	28	40	14:14	163
%	4.29	4.29	4.91	7.36	6.13	7.98	7.98	15.34	17.18	24.54	24.54	100.00

TABLE 9  
Frequency of bivalents at IM of PMC's of MachSaF<sub>1</sub> plants

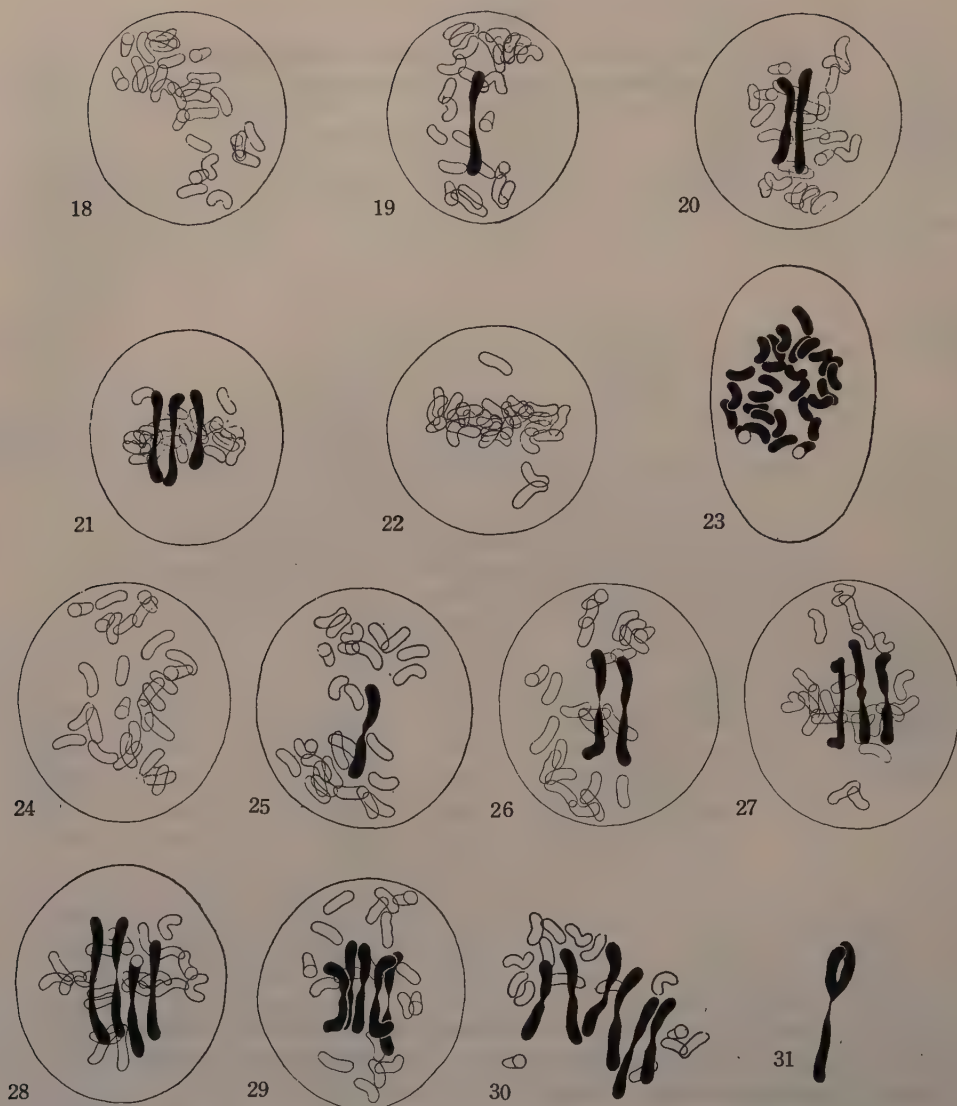
Individuals	Number of bivalents					
	0	1	2	3	Mode (%)	Total
MachSaF <sub>1</sub> -3	163	326	105	6	1 <sub>II</sub> (54.33)	600
" 4	146	331	104	16	1 <sub>II</sub> (55.17)	600
" 5	189	335	113	13	1 <sub>II</sub> (51.54)	650
" 7	186	362	85	17	1 <sub>II</sub> (55.69)	650
" 9	145	384	102	19	1 <sub>II</sub> (59.08)	650
" 10	168	424	131	27	1 <sub>II</sub> (57.20)	750
Total	1000	2162	640	98	1 <sub>II</sub>	3900
%	25.64	55.44	16.41	2.51	55.44	100.00

for the cytological research. 0~3 bivalents were found in PMC at the heterotypic metaphase in every individual case. The frequency of bivalents in PMC's of the F<sub>1</sub> plants is shown in Table 9.

As is evident from Table 9, the number of bivalents up to 3 was found in all the MachSaF<sub>1</sub> plants. The configuration of one bivalent appeared to be the mode for each of 6 individual cases. In all PMC's only the stick-shaped bivalents were observed. In this stage, no trivalent or tetravalent was observed.

In the heterotypic metaphase of PMC's, the equatorial plate consisting of all the univalents was observed, as well as that of the F<sub>1</sub>-type division as mentioned in MachScF<sub>1</sub> plants. The percentage of the formation of the plate for total number of PMC's was shown in Table 10.

As shown in Table 10, PMC's that show the F<sub>1</sub>-type division mixed with those that have the equatorial plate in one another, were occasionally observed.



Figs. 18-23. Meiosis in PMC's of MachSaF<sub>1</sub> hybrid, heterotypic division.  $\times 770$ .

18. 28 univalents scattered in spindle (MachSaF<sub>1</sub>-3). 19. Metaphase, side view, 1<sub>II</sub>+26<sub>I</sub> (MachSaF<sub>1</sub>-3). 20. do. 2<sub>II</sub>+24<sub>I</sub> (MachSaF<sub>1</sub>-3). 21. do. 3<sub>II</sub>+22<sub>I</sub> (MachSaF<sub>1</sub>-3). 22. Equatorial plate consisted of all univalents, side view, 3 chromosomes separated off the plate (MachSaF<sub>1</sub>-3). 23. do. polar view (MachSaF<sub>1</sub>-9).

Figs. 24-31. Meiosis in PMC's of MachSmF<sub>1</sub> hybrid, heterotypic division.  $\times 770$ .

24. 28 univalents scattered in spindle (MachSmF<sub>1</sub>-5). 25. Metaphase, side view, 1<sub>II</sub>+26<sub>I</sub> (MachSmF<sub>1</sub>-5). 26. do. 2<sub>II</sub>+24<sub>I</sub> (MachSmF<sub>1</sub>-5). 27. do. 3<sub>II</sub>+22<sub>I</sub> (MachSmF<sub>1</sub>-2). 28. do. 4<sub>II</sub>+20<sub>I</sub> (MachSmF<sub>1</sub>-4). 29. do. 5<sub>II</sub>+18<sub>I</sub> (MachSmF<sub>1</sub>-8). 30. do. 6<sub>II</sub>+16<sub>I</sub> (MachSmF<sub>1</sub>-8). 31. Y-shaped trivalent (MachSmF<sub>1</sub>-8).

TABLE 10

Occurrence of the F<sub>1</sub>-type division and formation of equatorial plate at the heterotypic division in PMC's of MachSaF<sub>1</sub> plants

Individuals	F <sub>1</sub> -type division		Formation of equatorial plate		Total (%)
	Number	%	Number	%	
MachSaF <sub>1</sub> -3	1518	96.44	56	3.56	1574 (100.00)
" 4	523	92.08	45	7.92	568 (100.00)
" 5	1702	98.10	33	1.90	1735 (100.00)
" 7	986	96.48	36	3.52	1022 (100.00)
" 9	1936	94.49	113	5.51	2049 (100.00)
" 10	2059	98.75	26	1.25	2085 (100.00)
Total	8724	96.58	309	3.42	9033 (100.00)

To sum up, the formation of the equatorial plate is considerably less (3.42%) than the F<sub>1</sub>-type division (96.58%). And the percentage of the formation of equatorial plate in PMC's of the F<sub>1</sub> corresponds to 1/3 that of MachScF<sub>1</sub> plants. The percentage of the fertility per spikelet in MachSaF<sub>1</sub> plants is 1.1, and this value also corresponds to 1/3 that of MachSaF<sub>1</sub> plants (Table 4). The percentage of the formation of equatorial plate and that of the fertility seem to have a certain active correlation with each other.

The distribution of chromosomes to opposite poles at the ana-telophase in heterotypic division proceeded at random, but in most cases, the number of chromosomes distributed to the poles was usually not so different for both poles.

In the tetrad stage, the tetrads consisting of 2~8 cells were observed in most cases. Those consisting of 4 cells were shown to be the mode (72.86%).

#### 4. *MachSmF<sub>1</sub> plants*

In this hybrid, 61 F<sub>1</sub> plants were raised, and 13 individuals were taken for cytological research. The number of bivalents found in one PMC at heterotypic metaphase was 0~3 in one individual, 0~4 in 7, 0~5 in 2 and 0~6 in the rest. The frequency of bivalents in one PMC is shown in Table 11.

As seen in Table 11, the mode of occurrence of bivalents was found to have the value 3 in one plant, 2 in one, 1 in 10, and 0 for the remaining one.

Throughout the whole 13 plants the configuration with one bivalent appeared to be mode.

In 6500 PMC's, 10 trivalents were observed, also bivalents at the heterotypic metaphase, but no tetravalent was found. The shapes of the trivalents were V- or Y-shaped.

The distribution of chromosomes to opposite poles at ana-telophase in heterotypic division proceeded at random and the proportions of 0:28~14:14 were observed.

In the tetrad stage, the tetrads consisting of 1~8 cells were observed.

TABLE 11  
Frequency of bivalents at IM of PMC's of MachSmF<sub>1</sub> plants

Individuals	Number of bivalents										Total
	0	1	2	3	4	5	6	*R	**III	Mode (%)	
MachSmF <sub>1</sub> -1	218	236	34	12						1 <sub>II</sub> (47.20)	500
" 2	161	171	107	51	10					1 <sub>II</sub> (34.20)	500
" 4	82	97	115	119	45	30	12	4	4	3 <sub>II</sub> (23.80)	500
" 5	187	238	62	12	1					1 <sub>II</sub> (37.60)	500
" 6	239	197	45	16	3					0 <sub>II</sub> (47.80)	500
" 7	198	221	59	19	3					1 <sub>II</sub> (44.20)	500
" 8	124	145	150	52	21	6	2	1		2 <sub>II</sub> (30.00)	500
" 10	154	181	89	50	22	4				1 <sub>II</sub> (36.20)	500
" 11	171	203	91	29	6			3	7	1 <sub>II</sub> (40.60)	500
" 14	140	217	104	34	4	1		1	7	1 <sub>II</sub> (43.40)	500
" 15	161	176	92	40	23	7	1	1	13	1 <sub>II</sub> (35.20)	500
" 16	214	221	49	14	2					1 <sub>II</sub> (44.20)	500
" 40	138	214	101	42	5					1 <sub>II</sub> (42.80)	500
Total	2187	2517	1098	490	145	48	15	10	31	1 <sub>II</sub>	6500
%	33.65	38.72	16.89	7.54	2.23	0.74	0.23			38.72	100.00

\* R is ring-shaped bivalent. \*\* III is trivalent.

Those consisting of 4 cells seem to be the mode (73.02%).

The bivalents up to 3 or 6 found in PMC's of the four F<sub>1</sub> hybrids mentioned above, —MachScF<sub>1</sub>, MachSVF<sub>1</sub>, MachSaF<sub>1</sub> and MachSmF<sub>1</sub>—, may have resulted from autosome synapsis between the chromosomes of A and B, or AB and D genomes of *T. Macha* as the mother plant, the fact according with the results from the cytological researches on the F<sub>1</sub> hybrids raised between Dinkel wheat and *Secale* by Aase [1], Kagawa and Chizaki [2], Kattermann [3], Kihara [4], Meister and Meister [5], Nakajima [6, 7, 8, 9, 10, 11], Thompson [15] and many other investigators.

### SUMMARY

In the present investigation, cytological and genetical researches have been made on the F<sub>1</sub> of the four combinations between *Triticum Macha* and each of the four species of *Secale* (*cereale*, *Vavilovii*, *africanum* and *montanum*).

The external characteristics of the F<sub>1</sub> plants resemble more closely the mother plant than those that are intermediate, though they represent external characteristics of both parents (Table 3).

At the ripening stage, the spikelets of MachScF<sub>1</sub> and MachSVF<sub>1</sub> are not brittle, while those of MachSaF<sub>1</sub> and MachSmF<sub>1</sub> are brittle and this characteristic must have been come from the original characters of *Secale* species as the pollen parent, for the mother plants are of the same in all these cases, with the difference only in the pollen parents.



The  $F_1$  plants were fertile by natural selfing in every combination, though not highly fertile (Table 4).

The number of chromosomes,  $2n$ , of  $F_1$  plants in all combinations was 28 which corresponds to the sum of the gametic chromosomes of the parents.

The number of bivalents at heterotypic metaphase of PMC's varied from 0~3 to 0~6 according to the combinations (Tables 5, 7, 9 and 11). These bivalents found in meiosis of PMC's of the four  $F_1$  hybrids might probably have resulted from autosynopsis between the chromosomes of A and B or AB and D genomes of *T. Macha* as the mother plant.

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# AN INVESTIGATION ON THE SCOPE OF A NUMBER OF PRE-TREATMENT CHEMICALS FOR CHROMOSOME STUDIES IN DIFFERENT GROUPS OF PLANTS

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AND

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## I. INTRODUCTION

Importance of pre-treatment methods as an aid to the study of chromosome structure has been amply demonstrated in recent years [1, 2, 3, 4, 5, 8, 9, 10, 12, 13, 14, 17, 18]. Specially due to the sustained effort carried out by different schools of research, a number of pre-treatment chemicals are at present known, which when suitably adjusted in techniques, help considerably in the clarification of karyotypes. Taking full advantage of these methods, different groups of plants have been cytologically investigated which were hitherto considered extremely difficult for such a study. Even species with very high chromosome numbers have been satisfactorily analysed from this aspect.

With the continued invention of a number of pre-treatment chemicals, necessity has been felt for a realization of the principle underlying their action. In absence of this understanding, even in spite of a number of techniques at hand, it is not possible to predict the suitability of a chemical for a clarification of the karyotype of a particular species. The entire procedure simply involves a series of random attempts and expecting the results on a chance basis.

Certain amount of researches has, however, been carried out on the principle underlying the mode of action of these pre-treatment chemicals. It has been shown that considerable changes in the viscosity of the plasma resulting nearly into its solidification is the principal effect of the pre-treatment chemicals [16]. As the plasma becomes solidified, pressure applied over the chromosomes at the time of smearing results into their wide scattering. Differential hydration undergone by the different chromosome segments effects a manifestation of the different segments of chromosomes—thus resulting into the clarification of the karyotype.

It has been worked out that this change in viscosity of a particular tissue can be brought out by causing any change in the surrounding medium, even with the application of distilled water [15]. This action of distilled water has been explained on the basis that even distilled water serves as a foreign medium for the nucleus of the plant tissue and as such a viscosity change can easily be effected. The degree of change in viscosity necessary for the clarification of karyotype may, however, be differentially caused by different chemicals. Therefore, species differ with respect to response to a particular chemical, as regards

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clarification of a particular karyotype. Such a suggestion implies that different chemicals are suitable for different groups of plants.

It is, however, not known yet as to whether this difference in manifestation of chromosome morphology by the different chemicals, depends to some extent on the total amount of chromatin matter present in the complement or it is attributable to some inherent characteristics of different families or even species of plants. Putting the problem more precisely, it may be stated that nothing is known yet as to whether different species with a common type of chromosome, say long or short, yield similar or dissimilar results following treatment with a particular chemical. Similarly, it is also not known as to how different species of the same family having different chromosome types respond to a particular chemical treatment. These are the points which are indeed worth investigation, as the issues mentioned above, if clarified, would indeed help in the choice of techniques before undertaking chromosome studies of any species, without necessarily depending on trials on a change basis.

In order to arrive at an understanding of these issues, the present work on the study of the effect of different pre-treatment chemicals on several species of two different families, one of Dicotyledons and one of Monocotyledons, has been planned. Different species under each family, where different types of chromosome complements are represented, have been chosen.

## II. MATERIALS AND METHODS

The following species of plants were used for this investigation:—

- |                |   |
|----------------|---|
| Amaryllidaceae | 1) <i>Crinum kunthianum</i> M. Roem.    |
|                | 2) <i>Pancratiun zeylanicum</i> Linn.   |
|                | 3) <i>Curculigo orchioides</i> Gaertn.  |
|                | 4) <i>Hemerocallis aurantiaca-major</i> |
| Umbelliferae   | 5) <i>Foeniculum vulgare</i> Gaertn.    |
|                | 6) <i>Hydrocotyle asiatica</i> Linn.    |
|                | 7) <i>Seseli indicum</i> W. and A.      |

The different pre-treatment chemicals tried were the following:

- 1) Aesculin
- 2) Oxyquinoline
- 3) Para-dichlorobenzene
- 4) Chloral hydrate
- 5) Colchicine
- 6) Coumarin
- 7) Phloroglucinol

Healthy root-tips of the different materials were treated with the different pre-treatment chemicals. Period of treatment, temperature and concentration of the chemicals were altered to find out that treatment which was optimum for bringing out the details of chromosome morphology (Table 1).

Root-tips after treatment with the chemicals were fixed and stained in a mixture of 2% Aceto-orcin and N/HCl (9:1) by heating for 3–4 seconds. The



TABLE 1  
Concentration, period and temperature of different pre-treatment trials

Chemicals	Concentrations used	Periods of treatment	Temperature trials
1) Aesculin	Saturated aqueous solution.	$\frac{1}{2}$ hr. to $2\frac{1}{2}$ hrs.	3-4°C to 14-15°C
2) Para-dichloro-benzene	a) Saturated aqueous solution. b) Half-diluted solution.	1 hr. to 4 hrs.	6-8°C to 14-15°C
3) Phloroglucinol	a) 0.005 Molar soln. b) 0.025 " c) 0.05 "	2 hrs. to 4 hrs.	
4) Coumarin	Saturated aqueous solution.	1 hr. to 4 hrs.	
5) Oxyquinoline	0.002 Molar soln.	1 hr. to 3 hrs.	
6) Colchicine	a) 0.1% Soln. b) 0.25% " c) 0.5% "	1 hr. to $2\frac{1}{2}$ hrs.	
7) Chloral hydrate	a) 0.5% Soln. b) 1% " c) 2% "	1 hr. to $2\frac{1}{2}$ hrs.	

materials were next kept in the mixture for 15-20 minutes and finally squashed in 1% Aceto-orcein. Over heating was avoided in every case. After smearing, slides were properly sealed.

No acetic-alcohol fixation procedure was followed after pre-treatment. This is because, on trials it was found that following a further acetic-alcohol fixation no significant differences in preparations were noticed.

### III. OBSERVATIONS

Results with the different chemicals show that with Amaryllidaceous species, Aesculin (saturated aqueous solution) was most suitable for clarification of details of chromosome. But in Umbelliferous species, an aqueous solution of Para-dichlorobenzene yielded best results with regard to somatic chromosomes. The detailed results are given below (Figs. 1-6).

#### 1. *Pancratium zeylanicum* ( $2n=48$ )

Nature of chromosomes—High number and mostly of medium size.

Pre-treatments with Coumarin, Oxyquinoline, Colchicine and Chloral hydrate at different temperatures and for different periods did not yield satisfactory results. Altered concentrations of these chemicals did not yield any better result. Para-dichlorobenzene and Phloroglucinol produced somewhat satisfactory results as regards scattering, staining and contraction of chromosomes. But secondary constrictions were not clear.

Best results were obtained with Aesculin (saturated aqueous solution) treat-



ment for 45 minutes at 3-4°C. Morphology of the chromosomes, staining scattering and contraction—all were found to be optimum at this treatment.

TABLE 2  
Effect of pre-treatment chemicals on the chromosomes of  
*Pancratium zeylanicum*

Treatments	Remarks
1) Aesculin (45 mts. at 3-4°C)	Optimum for chromosome morphology, scattering, staining and contraction.
2) Para-dichlorobenzene	Fragmentation at prolonged treatment and no scattering at short treatment.
3) Phloroglucinol	Secondary constrictions not clear.
4) Coumarin	Precipitation not good.
5) Oxyquinoline	Extreme contraction at prolonged treatment. Short treatment—no scattering.
6) Colchicine	Diplo-chromosomes at higher concentration.
7) Chloral hydrate	Lower concentration—no significant effect.

2. *Crinum kunthianum* ( $2n=22$ )

Nature of chromosomes—Long size and low number.

The optimum effect regarding the details of the chromosome morphology, scattering and staining was obtained with Aesculin (saturated aqueous solution for 30 minutes at 8-10°C). Results with Para-dichlorobenzene and Coumarin were somewhat satisfactory regarding scattering and staining. But secondary constrictions were not very clear. Prolonged treatment in these chemicals caused fragmentation and extreme contraction of chromosome arms.

TABLE 3  
Effect of pre-treatment chemicals on the chromosomes of  
*Crinum kunthianum*

Treatments	Remarks
1) Aesculin (30 mts. at 8-10°C)	Optimum for chromosome morphology.
2) Para-dichlorobenzene	Fragmentation at prolonged treatments. Secondary constrictions not sharp.
3) Coumarin	Scattering perfect but secondary constrictions not very sharp.

3. *Curculigo orchoides* ( $2n=18$ )

Nature of chromosomes—Low number; mixture of medium sized and short chromosomes.

The optimum effect was brought out by Aesculin treatment (saturated aqueous solution) for 45 minutes at 3-4°C.

TABLE 4  
Effect of pre-treatment chemicals on the chromosomes of *Curculigo orchoides*

Treatments	Remarks
1) Aesculin (45 mts. at 3-4°C)	Precipitation and scattering good; no granulation; staining bright. Primary and secondary constrictions sharp.
2) Para-dichlorobenzene	Granulation at lower temp. and swelling at higher temp.
3) Coumarin	Scattering perfect.
4) Oxyquinoline	Results not satisfactory, chromosomes pycnotic.
5) Chloral hydrate	
6) Phloroglucinol	

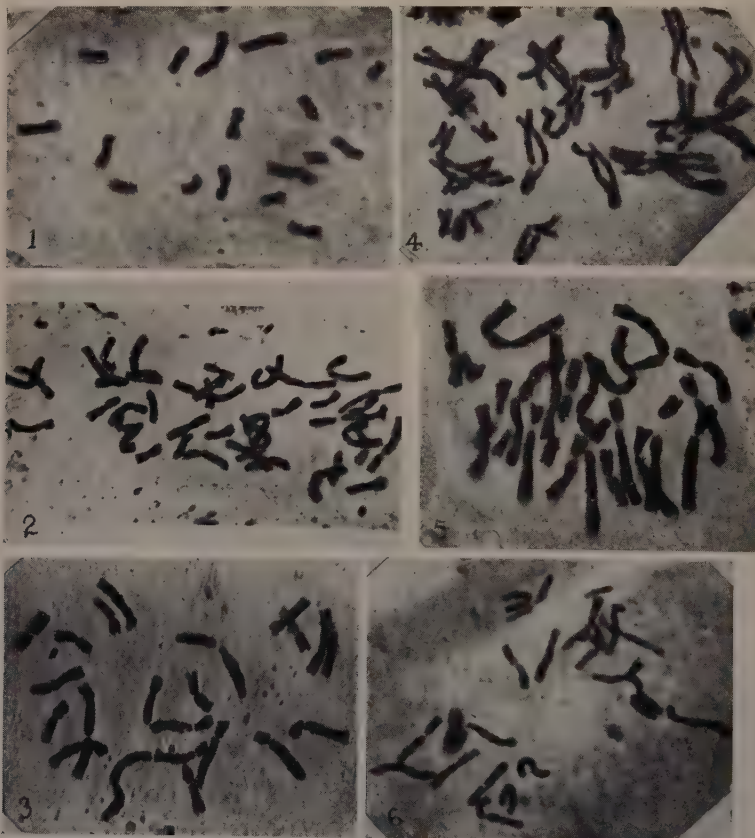


Fig. 1. Somatic metaphase of *Curculigo orchoides* after pre-treatment with Aesculin (45 mts. at 3-4°C). Fig. 2. Somatic metaphase of *Pancratiun zeylanicum* after Aesculin treatment (45 mts. at 3-4°C). Figs. 3-5. *Crinum kunthianum*. Fig. 3. Somatic metaphase after Aesculin treatment (30 mts. at 8-10°C). Figs. 4 and 5. Diplochromosomes and fragments after Aesculin (1 hr. at 3-4°C) and para-dichlorobenzene (4 hrs. at 8-10°C) treatment, respectively. Fig. 6. Somatic metaphase of *Foeniculum vulgare* after pre-treatment with Para-dichlorobenzene (2 hrs. at 10-12°C).

4. *Hemerocallis aurantiaca-major* ( $2n=22$ )

Nature of chromosomes—Low number and short size.

Coumarin and Phloroglucinol treatments yielded satisfactory results regarding scattering. Para-dichlorobenzene was found to be very effective in scattering and bringing out primary constriction regions. But the chromosome morphology with its details including the clarification of secondary constrictions came out clearly in Aesculin treatment for 30 minutes at 4-6°C. Colchicine and Chloral hydrate produced fragmentation and extreme granulation.

TABLE 5

Effect of pre-treatment chemicals on the chromosomes of  
*Hemerocallis aurantiaca-major*

Treatments	Remarks
1) Aesculin (for 30 mts. at 4-6°C)	Morphology clear; staining and scattering perfect.
2) Coumarin	Scattering perfect; morphology not clear.
3) Phloroglucinol	
4) Para-dichlorobenzene	Scattering perfect; primary constrictions sharp.
5) Colchicine	Granulation at higher concentration; at low concentration—no scattering.
6) Chloral hydrate	

5. *Foeniculum vulgare* ( $2n=32$ )

Nature of Chromosomes—Low number and long size.

In this species, optimum effect was obtained by treatment with Para-dichlorobenzene solution. Saturated aqueous solution was half-diluted with water and treatment with this dilute solution for two hours at 10-12°C produced best results. Other treatments were not satisfactory and generally clumping of chromosomes was the results.

TABLE 6

Effect of pre-treatment chemicals on the chromosomes of  
*Foeniculum vulgare*

Treatments	Remarks
1) Para-dichlorobenzene ( $\frac{1}{2}$ diluted soln. for 2 hrs. at 10-12°C)	Chromosome morphology absolutely clear. Staining and scattering perfect.
2) Aesculin	Heavy contraction and fragmentation at prolonged treatment.
3) Coumarin	Stickiness; staining faint; chromosomes pycnotic.
4) Oxyquinoline	

6. *Hydrocotyle asiatica* ( $2n=18$ )

Nature of chromosomes—Low number and short size.

Here, a saturated aqueous solution of Para-dichlorobenzene was found to be

most effective in bringing out the details of the chromosome morphology as well as scattering of chromosomes. With Aesculin, scattering and staining had been achieved but clarification of chromosome structure was not upto the mark. It did not respond well to treatment with other chemicals.

TABLE 7  
Effect of pre-treatment chemicals on the chromosomes of *Hydrocotyle asiatica*

Treatments	Remarks
1) Para-dichlorobenzene (saturated soln. for 2½ hrs. at 10–12°C)	Chromosome morphology very clear; scattering and staining perfect.
2) Coumarin	Staining very faint; scattering perfect.
3) Phloroglucinol	
4) Aesculin	Scattering and staining perfect. Chromosome morphology not clear.

7. *Seseli indicum* (2n=42)

Nature of chromosomes—High number and long size.

TABLE 8  
Effect of pre-treatment chemicals on the chromosomes of *Seseli indicum*

Treatments	Remarks
1) Para-dichlorobenzene (saturated soln. for 2 hrs. at 10–12°C)	Morphology clear; staining bright; no granulation. Secondary constrictions very sharp.
2) Aesculin	Fragmentation and granulation.
3) Coumarin	Scattering perfect; staining very faint.
4) Phloroglucinol	"
5) Colchicine	Granulation; staining faint.
6) Chloral hydrate	

TABLE 9  
Treatments which yielded best results for different species

Species	Chemical	Period of treatment	Concentration	Temperature
1) <i>Pancratium zeylanicum</i>	Aesculin	45 mts.	Saturated aqueous soln.	3–4°C
2) <i>Crinum kunthianum</i>	"	30 "	"	8–10°C
3) <i>Curculigo orchoides</i>	"	45 "	"	3–4°C
4) <i>Hemerocallis aurantiaca-major</i>	"	30 "	"	4–6°C
5) <i>Foeniculum vulgare</i>	Para-dichlorobenzene	2 hrs.	½ diluted saturated soln.	10–12°C
6) <i>Hydrocotyle asiatica</i>	"	2½ "	Saturated aqueous soln.	10–12°C
7) <i>Seseli indicum</i>	"	2 "	"	10–12°C



The optimum effect was brought out by Para-dichlorobenzene treatment for 2 hours at 10–12°C. In this treatment, chromosomes were found to be brightly stained with their morphology very clear. Secondary constrictions were very sharp. Other chemicals did not yield satisfactory results.

#### IV. DISCUSSION

Results obtained following treatment with different chemicals clearly indicate that the effect noted so far on different members of the same family is the same.

Of Amaryllidaceous species chosen, *Crinum kunthianum* is characterized by low number and long size of chromosomes, *Hemerocallis aurantiaca-major* by low number and short size, *Pancratium zeylanicum* by high number and mostly medium size and *Curculigo orchoides* ( $2n=18$ ) by a mixture of medium sized and short chromosomes. Of all the chemicals tried on these species at different temperatures and for different intervals, Aesculin is found to be the most suitable one so far as the clarification of karyotype is concerned. With other chemicals, contraction or at a slightly higher concentration fragmentation has been found to be the outcome.

Though Aesculin has been found to be suitable, in general, for all the species of this family, slight differences are, however, noticed as regards the temperature and time of treatment required by the different species for a clarification of their chromosome morphology. For example, optimum effect with Aesculin has been noted while applied for 45 minutes at 3–4°C in case of *Pancratium* and *Curculigo*. For *Crinum*, 30 minutes treatment at 8–10°C and for *Hemerocallis* 30 minutes treatment at 4–6°C are found to yield best results. In all the cases, however, a saturated aqueous solution of Aesculin has been found to give satisfactory results.

It therefore appears that all the different chromosome types, characteristic of the family Amaryllidaceae, respond more or less in a similar way to the effect of Aesculin. This possibly indicates that the cytoplasmic constitutions of different species of the same family have at least certain common characteristics which have enabled them to respond more or less in a homogeneous way to the chemical.

The slight difference in their effect regarding the time and temperature required may be due to the specific differences in the minute details of the plasmatic constitution of their cells. The *plasmatic constitution* is mainly emphasized as it is apparent that action of all these chemicals on chromosomes is indirect and the direct effect is exerted on the cytoplasm [vide 16]. The cytoplasmic changes cause consequent repercussion in chromosome structure.

In members of the dicotyledonous family Umbelliferae on the other hand, optimum effect, so far as the clarification of the karyotype is concerned, has been obtained following Para-dichlorobenzene treatment. In this family too, the species chosen for treatment are characterized by different types of chromosome complements. Of the species, *Foeniculum vulgare* is characterized by low number and long size of chromosomes, *Hydrocotyle asiatica* by low number and short size and *Seseli indicum* by high number and long size of the chromosomes.

It may be noted that though satisfactory effect as regards the clarification of chromosome details has been obtained in all these species following Parachlorobenzene treatment, species differ with respect to temperature, period of treatment and concentration of the solution for bringing out the desired results. Certain concentrations bring out drastic effect resulting into contraction of chromosomes, whereas others yield good reproducible results. All these minute differences of treatment are, obviously, attributable to differences in minor details of plasmatic constitution between species to species.

Summarising the data, it stands as thus—that different species of the same family more or less respond similarly to the effect of a chemical; on the other hand, species belonging to different families may differ with respect to their response. At the same time, though the general response among the species of a same family is more or less similar, minor differences are noticeable specially as regards the exact concentration, time and temperature necessary for treatment.

It has already been emphasized that in smears, the clarification of chromosome details is mainly dependent on the viscosity changes caused in the plasma. This change can be brought out by any disturbance in the cytoplasmic medium and as such even distilled water can bring out the effect [15]. All the chemicals, evidently, cannot bring out the same degree of viscosity change in all the species and as such different chemicals are generally effective for different species.

In addition, the present observation suggests that though species differ with respect to chemicals concerned, a good deal of similarity in the general response is present among the species of a same family. This fact may be considered as suggesting that the general plasmatic constitutions of different species of the same family are to some extent similar, which is responsible for their common type of response. It is quite likely that the genomic constitution of a species also controls the constitution of the cytoplasm. Naturally, as genomic constitution of one species is different from the other, minor differences in the cytoplasmic constitution are also expected. That is possibly the reason why a chemical, though effective, is to be employed in slightly different ways that is, either varying the concentration or temperature or the period of treatment in different species to bring out the optimum effect.

The above statement does not necessarily imply that a particular chemical suited for one family must not hold good for others. It may or may not give good results in other families. Optimum response can be obtained in a number of families following a common chemical treatment. But what is emphasized here is that, species of the same family behave in a similar way. This understanding is highly essential in order to avoid trials on a random basis.

Similar types of experiments on other families of plants are very necessary to find out whether the results obtained in the present set of experiments hold good for others. Once it is established, researches on chromosome study will be considerably helped, as attempts with different chemicals to secure good results on a chance basis can considerably be avoided.

## V. SUMMARY

1. Seven different pre-treatment chemicals viz., Aesculin, Para-dichlorobenzene, Coumarin, Oxyquinoline, Phloroglucinol, Colchicine and Chloral hydrate were tried on plants of two different families, one belonging to monocotyledonous and the other to dicotyledonous groups viz., Amaryllidaceae and Umbelliferae.

2. Different species, in which the chromosome complements of varying types are present, were chosen for treatment. For example, low number and long size is represented in species of *Crinum kunthianum* and *Foeniculum vulgare*, high number and long size in species of *Seseli indicum*, high number and medium size in *Panocratium zeylanicum*, low number and short size in *Hemerocallis aurantiaca-major* and *Hydrocotyle asiatica*, and low number and a mixture of medium-sized and short chromosomes in *Curculigo orchioides*. It has been desired to find out whether different species of the same family respond similarly or not, which would give an understanding of the basis of their action.

3. With Amaryllidaceous species, irrespective of the type of chromosome complements they possess, Aesculin has been found to be the best for clarification of the details of chromosome structure, whereas with Umbelliferous members, Para-dichlorobenzene has been found to be most suitable in all the species.

4. The similar response of the members of a particular family with different types of chromosomes is an indication that their cytoplasmic constituents too have certain common characteristics. The response is, therefore, due to common characteristics of the family and not dependent on the total amount of chromatin matter or the types of chromosomes the species possess.

5. It has been emphasized that if a chemical suitable for the members of a particular family can be found out, trials with different chemicals on a chance basis can be avoided.

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# SEX EXPRESSION AND DETERMINATION IN SPINACH<sup>1)</sup>

## I. GROWTH HABIT AND ITS SEX-LIMITED INHERITANCE<sup>2)</sup>

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### I. INTRODUCTION

In nearly all dioecious organisms, the sexual differentiation appears not only in the sexual organs but also in the vegetative ones. Spinach, for example, is one of the dioecious crops, and it represents one of these cases. That is to say; the female plant is agronomically superior to the male plant in having heavier green yield on account of its luxuriant nature resting with the longer growth period and the later bolting time [9, 11, 12, 13], containing in more vitamins A and C [14], and also in being better in the taste mainly owing to the lower content of oxalic acid [23]. In a spinach field it is therefore quite important and practical to grow a population with only one sex, female. Nevertheless, the detection of sexuality is either impossible or very difficult at any stage of the growing season before bolting in this plant as well as in any other dioecious crops. In those crops the intense interest of breeders is to establish some practical procedure of sex control, on which, up to date, useful data have not so much accumulated. Since 1953 the present work on spinach has been carried out along this line.

As can be seen in common with general dioecious organisms, the sexual characters of spinach comprise two kinds of organs in appearance. One is named the "primary sexual characters" representative of the sexual organs, male and female flower, and the other, the "secondary sexual characters" of the vegetative organs mentioned already. Here, these two characters were conveniently called the "sexual" and "growth habit", respectively. Some differences regarding them were certainly associated with the sexuality, maleness or femaleness. But such an association of characteristics to the sexuality was not always found in some cases of intersex plants, i. e., some female-like plants in the sexual habit were at times of the male-like pattern in the growth habit, and vice versa. In the present work the genetic mechanism of sexuality has been examined and discussed by separating data on each of two sexual characters. This paper deals with only the growth habit.

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2) Dedicated to Prof. Hajime Matsuura and Prof. Yukio Yamada celebrating their Sexagenary birthdays.

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## II. MATERIALS AND METHODS

Spinach used is of a Japanese native race furnished from the Sakata Seed Co., Ltd., Chigasaki, Japan, by favour of Dr. T. Eguchi, in 1953. This race belongs to the Asiatic group of the prickly variety [8].

Spinach was grown under two different conditions of culture, box and field. In the box culture the size of box was 60 cm long, 45 cm wide and 12 cm height. Soil sterilized by burning was filled in boxes, and then seeds were planted 5 cm apart, totaling 99 plants per box. In winter these boxes were placed in the greenhouse with the day-length adjusted to make the flowering and the seed-setting possible. The field culture was used for the main purpose of determining the segregation ratio on the growth habit in the given population.

## III. RESULTS OF OBSERVATIONS ON THE GROWTH HABIT

### 1. *Classification into three patterns*

For any characteristics as to the growth habit no sexual differentiation was observed at the foliage stage before the bolting time. But from the time of bolting onwards the differentiation gradually become clearer according to the elongation of the bolting stalk, and finally completed at anthesis. Differences of characteristics concerned made it possible to classify the growth habit into three patterns. With respect to the sexual habit, one of them was usually female, and the remaining two male.

a) *The bracted male-pattern*, "B♂". This was one kind of male plant. The bolting time was earlier than that of the other patterns by several days (Fig. 1-a). Each internode of the stalk seemed to gradually elongate in accordance with the progress of bolting. A cluster with several sessile flowers arose on a long peduncle well-developing from each axilis of the upper nodes of the stalk. All of the cauline leaves arising from the nodes with the flower cluster were found to degenerate into a calyx-like bract. As a consequence the upper part of the stalk including those flowers and bracts gave rise to a compound spike, shedding pollen grains in abundance at anthesis. This is described as the "Bracted Male Pattern" in an abbreviation of "B♂".

b) *The leafy male-pattern*, "L♂". This was another kind of male plant, which is sharply distinguished from the former B♂ pattern by later elongating the flower stalk, and by developing a non-degenerating cauline leaf from every flower-cluster-bearing node (Fig. 1-b). Each of the axillary flower-clusters arose on a short peduncle from each upper-node with a well-developing cauline leaf. The clusters did not give rise to a compound spike, but each of them was separated from the other by a cauline leaf. On the whole, the characteristics of organs, the plant size and its luxuriance were rather similar to those of the next pattern of the female plant. But the former differed from the latter in its detailed growth and sexual habits; in less-developing of the cauline leaves, in having a short growing-season and so on. Generally speaking, this seemed to be an intermediate form between the B♂ and ♀ patterns, and may be describ-



Fig. 1. Three patterns of the growth habit occurring in the prickly race of Japanese spinach, of which *a* in the photograph is the *Bracted male* (B♂), *b* the *Leafy male* (L♂) and *c* the *Female* (♀).

ed here as the "Leafy Male-Pattern" to which an abbreviation "L♂" is given.

c) *The female pattern*, "♀". Characteristics concerning the shape of cauline leaf and the length of peduncle closely resembled those of the L♂ pattern. But this was markedly different from the latter, L♂, in having a longer growing season, a less degree of discoloration of the flower stalk, and a two-divided perianth (Fig. 1-c).

## 2. Comparison of the bolting and flowering time

A comparison was made for both cultures, winter and spring. In winter culture, plants were sown in a box on December 24, 1954, and received a 9-hour day-length in the greenhouse, at which a temperature of approximately 20°C was held until February 9, 1955. From that day, under the same condition of greenhouse, they were treated with a 24-hour day-length under a compensation



of the electric light to bolt the flower stalk from the rosette plant. The flower forming date appearing on the stalk was continually recorded as an index of the bolting or flowering time, from February 19, 1955. Results obtained are illustrated in Fig. 2, indicating that a marked difference exists between these three

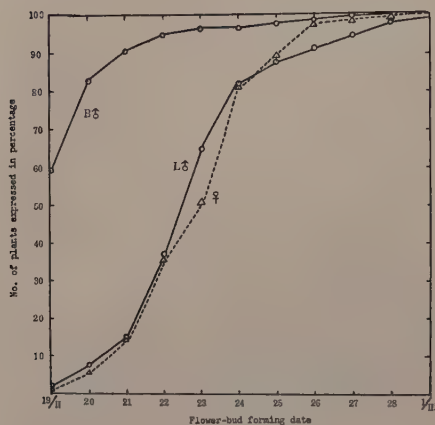


Fig. 2. Flower-bud forming time of Japanese prickly spinach under the winter culture. Symbols illustrated, B♂, L♂ and ♀, are given for the three patterns of the growth habit, *Bracted male*, *Leafy male* and *Female*, respectively.

patterns. On the first day of observation, about 60 percent of the B♂ plants had already bolted the flower stalk, forming a compound spike. In the other two patterns, L♂ and ♀, plants reached a similar bolting stage about 4 days later on the average.

In spring culture, seeds were planted on April 14, 1958 in a field. The date at which the flower stalk began to elongate approximately 3 cm was recorded as the bolting time. The average date was  $\text{May } 23.7 \pm 0.3$ ,  $\text{May } 24.3 \pm 0.5$  and  $\text{May } 22.6 \pm 0.3$  for B♂, L♂ and ♀, respectively. Similarly, the date of anthesis was  $\text{May } 28.8 \pm 0.2$ ,  $\text{May } 31.5 \pm 0.6$  and  $\text{May } 28.7 \pm 0.3$ . These data indicate that any differences among them are statistically not significant.

Summing up, a marked sexual differentiation regarding the bolting or flowering time should be expected to

appear under winter culture, but not under spring culture, so far as the present data are concerned.

### 3. Comparison of other characteristics concerned

In addition to the above mentioned characteristics, there were also other several characteristics to strongly support the classification into three patterns of the growth habit. The calyx-like bracteole, for example, was lacking in the B♂ flower, somewhat developed in the L♂ flower, and well-developed in the ♀ flower. All of the B♂ plants were certainly male, neither female nor intersex, always bearing male flowers only. Although it was a rule that the L♂ and ♀ patterns are male and female in the sexual habit, respectively, there were often some sex-reversals and intersexes occurring in their populations. The bracteole in the female flower from the ♀ pattern persisted until ripening of the fruit, becoming enlarged and hard, enclosing the seed, and finally forming the fruit coat. The seeds were covered with a thick coat conspicuously horned on the opposite side, giving rise to the so-called "Prickly Seed". The seeds from the female flower of the L♂ pattern were not, entirely, enclosed with a rudimentary coat, developing into the so-called "Naked Seed". The characteristics of perianth had however an opposite relationship against the persistent



bracteole; the most remarkable in the B♂ flower consisting of 4 or 5 petaloid lobes, but rudimentary in the L♂ flower and lacking in the ♀ flower.

Results on 10 characters concerned are given in Table 1. Considering all characters listed, a linear sequence of three patterns will be given as the "B♂-L♂-♀" in relationship to the sexual differentiation.

TABLE 1  
Characteristics of three patterns of the growth habit

Character	B♂	L♂	♀	Comparison
1. Cauline leaf	bract-like, degenerate	leafy, prominent	leafy, most prominent	$B♂ \ll L♂ = ♀$
2. Peduncle	long	short	short	$B♂ \gg L♂ = ♀$
3. Bolting time	early	late	late	$B♂ < L♂ = ♀$
4. Growth period	short	mediate	long	$B♂ < L♂ < ♀$
5. No. of leaves	fewer	many	most	$B♂ \ll L♂ \leq ♀$
6. Green yield (luxuriance)	less	more	most	$B♂ \ll L♂ \ll ♀$
7. Bracteole	lacking	somewhat developed	well-developed	$B♂ \ll L♂ < ♀$
8. Fruit coat	lacking	rudimentary	conspicuous	$B♂ \ll L♂ < ♀$
9. Perianth	well-developed	rudimentary	lacking	$B♂ > L♂ > ♀$
10. Intersexuality	none, with ♂ flowers only	presence, with ♂ often ♀ & ♂ flowers	presence, with ♀ often ♂ & ♀ flowers	$B♂ \ll L♂ = ♀$

With respect to the variability of character concerned, another one of the most interesting sexual features was found among three patterns. Namely, a continual variation for any character concerned usually existed between the L♂ and ♀ patterns, while a discontinuity of variation existed between the B♂ and L♂ or ♀. Naturally, there were many intermediate types concerning the sexual or growth habit in either patterns, L♂ and ♀. Despite of the absence of bi-sexuality in the B♂ plants, the hermaphrodite or sex-reversal monoecious flowers often appeared in some L♂ and ♀ plants, resulting in intersexuality in the sexual habit. It may thereby be said that the B♂ pattern should have more a remote relationship to the other two, L♂ and ♀, than that between the latter two in a viewpoint of the sexual differentiation.

#### IV. RESULTS OF EXPERIMENTS ON THE SEX-LIMITED INHERITANCE

##### 1. *Effect of the mass-selection of the L♂ pattern on the descendant population*

Of the first generation in the box culture an original population from the Sakata Seed Co. including a total of 1,574 plants was of the typical growth habit of the prickly Japanese spinach. In fact, the sexuality showed a ratio of 47 male to 53 female in the percentage expression, corresponding to the theoretical

ratio of 1:1. Further, out of 47 percent of male plants, 23 percent was of the B♂ pattern and the remaining 24 percent of the L♂ one, giving also an approximately equal number for the male growth habit. In this population one box with 99 plants was chosen, in which all B♂ plants were then discarded, and isolated from the other boxes before anthesis in order to prevent the pollen contamination. The plants remaining in this box should logically be expected to be a population with only the two patterns, L♂ and ♀, other than B♂.

From the next S<sub>1</sub> generation resulting in such a selection 303 plants were grown in the greenhouse, of which 150 plants were female and the remaining 153 male, agreeing with the normal sex-ratio. Most of the male plants were L♂, but there was in addition an exception of about 6 percent of the total plants, giving the actual numbers of 20 B♂ and 133 L♂ in the total of 153 male plants. At the present status of the experiment it remains unanswerable to ascertain whether an occurrence of these exceptional B♂ plants depends either on the pollen blending from some other B♂ plants or on some genetical causes in its population such as the crossing over of the gene or genes.

TABLE 2  
Genetical effect of two mass-selections on the L♂ pattern appearing in three successive generations

Generation	Culture	B♂	L♂	♀	Total
S <sub>0</sub>	greenhouse	364(23.1)	382(24.3)	828(52.6)	1,574(100.0)
S <sub>1</sub>	greenhouse	20 (6.6)	133(43.3)	150(49.5)	303(100.0)
S <sub>2</sub>	greenhouse	—(—)	20(54.0)	17(46.0)	37(100.0)
	field	1(0.2)	277(46.6)	316(53.2)	594(100.0)
	Subtotal	1(0)	297(47.1)	333(52.8)	631(100.0)
Total		1,197(47.1)		1,311(52.3)	2,508(100.0)

Note: The abbreviation under the 1st column, S<sub>0</sub>, S<sub>1</sub> or S<sub>2</sub>, means the generation of no, one or two mass-selection made for the B♂ pattern, respectively. All numerals without the parenthesis are the actual number of plants observed, and all ones within the parenthesis are of the percentage expression for the actual number counted.

The same selection was repeatedly made for one box of the S<sub>1</sub> generation. Seeds obtained were planted under both cultures, field and greenhouse. Results are arranged in Table 2, indicating that almost all of the male plants, of which the total number was 297 in the S<sub>2</sub> generation, were detected to be the L♂ pattern, but only one plant from the field culture to be the B♂ one. In this case no pollen contamination was considered, because the B♂ plants in the S<sub>1</sub> box treated were too few in number, as already mentioned, to make any failures on such a selection.

## 2. Crossing experiments on the male growth patterns

Some typical plants of the male growth patterns, B♂, L♂ and L♀, of which



Fig. 3. Representation of the male pattern appearing in two  $F_1$  populations, corresponding to the male pattern used as the pollen parent; the bracted pattern ( $B\sigma$ ) at the left side and the leafy one ( $L\sigma$ ) at the right side.

TABLE 3

Segregations for the growth habit in the  $F_1$  populations from 50 crossing combinations of three patterns used as the pollen parent,  $B\sigma$ ,  $L\sigma$  and  $L\phi$

Year observed	Pattern of pollen parent	No. of crossing combinations	Relationship between both parents	No. of segregated plants			
				$B\sigma$	$L\sigma$	$\phi$	Total
1956	$B\sigma$	14	unrelated	63	—	79	142
1957	$\{B\sigma$	4	unrelated	19	—	20	39
	$B\sigma$	5	sibmating	82	—	69	151
Subtotal	$B\sigma$	23	—	164	—	168	332
1956	$L\sigma$	11	unrelated	1	107	97	205
1957	$\{L\phi$	7	unrelated	—	57	56	113
	$L\sigma$	9	sibmating	—	131	130	261
Subtotal	$L$	27	—	1	295	283	579
Total		50	—	460		451	911



the last pattern,  $L\text{♀}$ , was designated for the bisexual plant belonging to the  $L\text{♂}$  pattern, were used as the pollen parent to cross with the female plants. All  $F_1$  populations from these crosses were grown in the field in 1956 and 1957. The segregation data obtained are represented in Table 3 and Fig. 3.

The  $F_1$  populations obtained from 50 crossing combinations were composed of 911 plants in total, of which the sexuality could be recognized to be normal, actually consisting of 460 male and 451 female plants. As can obviously be judged from Table 3, all of  $F_1$  male plants were always  $B\text{♂}$  when any one of the female plants was crossed with the  $B\text{♂}$  pollen parent, and also they were all  $L\text{♂}$  when their pollen source came from the  $L\text{♂}$  pattern. Contrary to these cases, only one out of the total of 460 male plants was however observed to be  $B\text{♂}$ , differing from the growth habit of its pollen parent. Despite that there was only one unexpected plant, like some cases of the selection experiment, it may be concluded with certainty that the growth pattern of the male plants appearing in the  $F_1$  population entirely agrees with that of the pollen parent used in their crossings, which is considered as a logical basis of the one-side inheritance. It is thereby quite natural that the characteristics of  $B\text{♂}$  or  $L\text{♂}$  should transmit from male to male in the progressive generation, indifferent to the female characteristics.

Table 3 bears also another evidence on the segregation of the male growth pattern that no difference exists in the following three components in the crossing combinations: (1) years, 1956 and 1957; (2) relationship between both parents, related (sibmating) and unrelated; and (3) difference of the sexual habit within the same growth habit,  $L\text{♂}$  and  $L\text{♀}$ . It therefore seems highly probable that these components should all be independent upon the present segregation of the growth patterns,  $B\text{♂}$  and  $L\text{♂}$ .

### 3. Genetic interpretation on the data

In order to account for all of the foregoing data on the mass-selections and crossing experiments, there is need of reconsidering the following three assumptions:

a) *The dominant gene locating on an autosome.* Granting the presence of an autosomal gene which is able to control only the male growth habit, indifferent to the female one, the gene must be simple and dominant in its nature to take a marked effect on the mass-selection. Further, a conditional basis must logically be accepted in this case that the male pattern differing from that of pollen parent should appear in some progenies, even though it is very few in number, from the mass-selection as well as from the hybrid, without considering the contamination of foreign pollens. Now under such a condition, one allele,  $A$  for  $B\text{♂}$  and  $a$  for  $L\text{♂}$ , will be regarded as the most suitable assumption. Then the frequency of the gene,  $A$ , possessing in the  $S_0$  population concerned is calculated to be 0.285, according to Hardy-Weinberg's formula. After carrying the mass-selection into practice, the remaining male plants must be all  $L\text{♂}$ , being expected to have the genotype of a homozygous  $a/a$ . Thus the  $B\text{♂}$  plants occurring in the next  $S_1$  population must be expected to all come from only the



female parent. Then the  $B\delta$  frequency is to be 0.131, and the  $L\delta$  frequency to be 0.715. Similarly, the male frequency in the  $S_2$  population was computed to be 0.068 for  $B\delta$  and 0.932 for  $L\delta$ . The ratio of the deviation between the expected and observed numbers to the standard error,  $d/\delta$ , was 7.35 and 4.42 for the  $S_1$  and  $S_2$  population, respectively, both giving a probability of almost zero. The data must therefore be considered as disagreeing with such an assumption. It seems that the present data cannot be interpreted by assuming any gene located on any autosome.

b) *The dominant gene locating on the X chromosome.* Not any heteromorphic chromosomes connected with the sexuality could be observed in either the mitotic or meiotic complement of chromosomes [17]. But the conclusive evidence to confirm that the XY type of the sex chromosomes is male, and the XX type female was gained from two cytogenetical data made by the authors. Namely, (1) the sex ratio of 3 male to 1 female always existed in the self-pollinated progenies of the bisexual " $L\delta$ " plant and its progeny test showed that one third is homozygous for the Y factor and the remaining two third heterozygous for it [16] and (2) a  $3n$  population from the cross between the  $4n$  male and the  $2n$  female plant consisted of 7 male and 1 female plants (unpublished).

All of the present data show that the appearance of male patterns,  $L\delta$  and  $B\delta$ , should be conditioned by only the genic constituents from the male parent, not female, usually corresponding to the growth pattern of male parent in the preceding generation. It is a well-known fact that the X chromosome in the male plant is always brought from the female side in the preceding generation. If the present assumption were adopted to the present data, the genetic effect of the male pattern upon a given population would just appear at least one generations later, irrespective of the dominancy of the gene concerned. Such an assumption is merely contrary to the fact.

c) *The gene or genes locating on the Y chromosome.* As already demonstrated, the male growth habit in the population,  $B\delta$  or  $L\delta$ , is transmitted from only the male side in the preceding generation, nothing from the female parent. Here, a hypothesis of the Y-limited inheritance that two patterns,  $B\delta$  and  $L\delta$ , are controlled by the gene or genes locating on the Y chromosome in the male plant can be considered to fit in with the present data. Against this hypothesis a few exceptions were recognized to occur in a few cases, but it may be believed that they should be caused by other unexpected factors such as pollen contamination from foreign populations, and the possible crossing-over between the X and Y chromosomes.

If the present hypothesis is accepted as correct, two kinds of the Y chromosome will usually be present in populations of Japanese spinach, one of which carries the genetic factor governing the  $B\delta$  pattern and the other the  $L\delta$  pattern, being called the  $Y^B$  and  $Y^L$  chromosome, respectively. The male plant must then have a complement of sex-chromosomes consisting of either  $XY^B$  or  $XY^L$ . In fact, of the male plants representing approximately 47 percent in the total of the  $S_0$  population, 23 percent can therefore be said to be  $XY^B$  and 24 percent

to be  $XY^L$ . In other words these two frequencies of the male patterns,  $B\delta$  and  $L\delta$ , should be those of occurrence for the Y chromosome itself,  $Y^B$  or  $Y^L$ , in this population. Generally, in the given population the  $L\delta$  plants with  $Y^L$  should increase when the  $B\delta$  plants with  $Y^B$  decreased, and vice versa, always keeping a constant ratio of an equal number for the sexuality, male and female, in the total plants. The other conclusive evidence could be further given from the crossing data, indicating that the growth pattern of the  $F_1$  male plants entirely agreed with that of their pollen parent in every cross. Therefore no other assumptions except the sex-limited inheritance seem to be able to account for the present data.

## V. DISCUSSION

The present classification of the growth habit into three patterns,  $B\delta$ ,  $L\delta$  and ♀, apparently agrees with that first announced by Rosa [12], pure male, vegetative male and pure female, and later adopted by Japanese workers [3, 4, 9]. Furthermore, to these three, Sugimoto [15] and Katayama et Shida [7] added one type named the "partially vegetative male". According to them, the last type is an intermediate form between the pure and vegetative males. This type may however be considered as a derived form of the  $L\delta$  pattern rather than an independent type. Actually, in consequence of the confusion of the segregation due to admitting this type, Sugimoto [15] could not draw the same conclusion with the authors on the inheritance of the male growth habit.

The present comparison of ten characters in connection with the growth habit is on the whole similar to those made by Rosa [12], Saitô [13], Sugimoto [15] and Murayama [9]. But it essentially differs in details, in showing a clear independence of the  $B\delta$  pattern to the others not only in the growth habit but also in the sexual habit, especially in the complete absence of intersexuality and the degeneration of bracteole (Table 1). In the  $B\delta$  pattern, Eguchi et Ichikawa [3] and Murayama [9] found some intersex plants but in all present cases with approximately 1,000  $B\delta$  plants growing under various conditions, cultures, seasons, temperatures and day-lengths, no intersexuality was observed in the  $B\delta$  pattern at all. As for the flowering date, under autumn or winter culture the  $B\delta$  pattern was 4 days earlier than the others, agreeing with that by Murayama [9] showing an earliness of 7-8 days. Under spring culture any difference was however not recognized among the patterns, corresponding to the result by Saitô [13]. The earlier flowering time should, like the other characteristics, be also characteristic of the  $B\delta$  pattern, of which some resemblances appearing under some conditions may be due to the specific response of anthesis on the environmental factors, as seen in common with the general quantitative characters of plant. These data may warrant a conclusion that the sexual differentiation of the  $B\delta$  pattern towards maleness is the most prominent so as to show the discontinuous variation of characters concerning both habits to the other patterns.

Since the male plant is well-known as the XY type of sex-chromosomes [5,

16] the two patterns of male, B♂ and L♂, could be genetically proved to be a case of the Y-limited inheritance. It was therefore pointed out already that there should be two kinds of the Y chromosome, one of which is "Y<sup>B</sup>" governing the B♂ pattern and the other "Y<sup>L</sup>" governing L♂. Making a complete discarding for one of two male patterns in a given population, only the other one should appear in the next generation. In fact, there were a few exceptions to this rule in a very few cases. Zoschke [23] reported that in all his mass-selections for the European spinach an unexpected exception constantly appears at a rate of several percent. This percentage is higher than in the present experiments. The occurrence of these exceptional plants should mostly be dependent on an incomplete management of the pollen isolation, although a part of them should rest with the genetical basis such as the crossing over.

The sex-limited inheritance was demonstrated by the F<sub>1</sub> data, indicating that the exceptional plants did not occur in 49 out of the 50 F<sub>1</sub> combinations, although there was only one exceptional plant in the remaining one F<sub>1</sub> combination. Sugimoto [15] pointed out that the F<sub>1</sub> male plants pollinated with the vegetative male plant (L♂) are all "vegetative male" as expected, whereas those with the pure male plant (B♂) segregate two patterns (B♂ and L♂) into various ratios contrary to the expectation, disagreeing with the present data. Those his cases may be interpreted by a possible supposition that his judgement is correct for the L♂ pattern and wrong for B♂, and if so, his B♂ plants used as the male parent must really be of an admixture of two patterns, B♂ and L♂. The sex-limited inheritance in accord with the present case has in some details been studied in two limnetic fishes, *Lebistis* [20, 21] and *Apocheilis* [1, 2, 22]. Since then, several instances dealing with *Melandrium*, *Drosophila*, *Lymantria* and mankind have been found to be completely parallel with those of Winge and Aida. In spinach two patterns of the male growth habit can be demonstrated as a case of the sex-limited inheritance in the plant kingdom.

Owing to the sex-limited inheritance it is quite natural that in the male side of the given population a decrease of one pattern, L♂ or B♂, is certainly accompanied with an increase of the other one, B♂ or L♂, and sum of two patterned male plants keeps a genetic equilibrium in sexuality equal to the half number of total plants in the population. Whether any one of two patterns, B♂ or L♂, is dominant over the other one, L♂ or B♂, or controlled by any pairs of genes are as yet unknown. It was also not ascertainable which one of six chromosome pairs is the sex chromosome, because general peculiarity of the sex chromosome, especially heteromorphic pairing and heteropynosis, was never observed in any one of spinach chromosomes. Nevertheless, it is reasonably understood that there should be at least two types of the Y chromosome, Y<sup>B</sup> and Y<sup>L</sup>, of which on each one, the determining factor for B♂ or L♂ is located. With respect to the sex-chromosomal constitution, three kinds of combinations, XY<sup>B</sup>, XY<sup>L</sup> and XX, should therefore exist for plant, according to which a plant is of a pattern, B♂, L♂ or ♀, respectively.

In addition to these male and female plants, some bisexual plants were often met with in the spinach field. Westergaard [19] named the "subdioecious



type" of dioecism to such a intersexuality, which is well-known to be most common in the dioecious plant kingdom. In spinach occasional bisexual plants occurred in both sexes,  $L\sigma$  and  $\varphi$ , although they never appeared in  $B\sigma$ . These two intersexualities coming from  $L\sigma$  and  $\varphi$  patterns could be distinguished to each other from their growth habit and chromosomal constitution [16, 18]. One with a few female or hermaphroditic flowers in the male flowered cluster is called "subandroecious" [19] agreeing with the  $L\sigma$  pattern in the growth habit. The other with occasional male or hermaphroditic flowers in the female flowered cluster is called "subgynoecious", corresponding to the  $\varphi$  pattern. The sex expression in both intersexual plants was found to be strongly influenced by some environmental factors, particularly temperature, day-length and their combination.

In conclusion, it seems probable that the  $Y^B$  chromosome should carry a strong male-determining factor, which is not affected by any genetic factor controlling the intersexuality, always giving rise to only the male, whereas the  $Y^L$  chromosome should bear a weak male-determining factor affected by the intersex gene, sometimes resulting in an occurrence of intersexuality belonging to the  $L\sigma$  pattern. Thus the  $B\sigma$  pattern can also be designated the "strong male", which is characterized by the distinct male peculiarity of characters concerned. The  $L\sigma$  pattern can be called the "weak male" characterized by the rather female-like peculiarity in both habits, sexual and growth. A phylogenetical sexual relationship of  $L\sigma$  to  $\varphi$  will therefore be assumed to be much closer than that of  $B\sigma$  to  $L\sigma$  or  $\varphi$ . The morphological differentiation of both Y chromosomes,  $Y^B$  and  $Y^L$ , was not so prominent in appearance that they were unable to separate not only from each other but also from the X chromosome.

General speaking, the occurrence of the  $B\sigma$  pattern is most frequent in the native varieties and less in the improved ones [6, 8]. In an oriental prickly variety native to the mountainous region in Central Nepal, for example, the  $B\sigma$  pattern is too predominant to find the  $L\sigma$  pattern [10]. A race comprising  $B\sigma$  plants with the  $Y^B$  chromosome will then be considered to be more primitive and more ancestral in the evolutionary origin of spinach than the other race containing  $L\sigma$  plants with the  $Y^L$  chromosome. A  $B\sigma$  plant sheds the abundant pollen grains, while an  $L\sigma$  plant a bit of them. Further, anthesis is proteandrous in  $B\sigma$ , and simultaneous in  $L\sigma$  with the  $\varphi$  pattern.

From a given race with both male patterns,  $B\sigma$  and  $L\sigma$ , a new line with only one male pattern will easily be recovered by carrying only one selection or crossing based on the Y-limited inheritance of the growth habit. Regarding the growth habit concerned, such a simple method for obtaining the recovered line is therefore worthy of breeding consideration.

## VI. SUMMARY

The present paper deals with the morphological and genetical study on the secondary sexual characters of the Japanese prickly spinach. These characters are named the growth habit for the convenience of description. The findings



obtained are summarized as the following:

1) From the morphological viewpoint, the growth habit was classified into the three patterns, bracted male (B♂), leafy male (L♂) and female (♀), from a comparison of ten characters concerned. Considering the degree of sexual differentiation on these characters, the three patterns were reasonably considered to be arranged in a sequence of "B♂—L♂—♀".

2) From the phylogenetical viewpoint, the B♂ pattern was assumed to be the most primitive form of male, representing the remarkable male peculiarity in all characters, whereas the L♂ pattern to be a secondary form, often preserving the female-like peculiarity in some characters as yet. The intersexuality was not observed in B♂ but often in L♂ as well as in ♀. Therefore it seems that the relationship of B♂ to L♂ is more remote than that of L♂ to ♀, and the L♂ pattern is intermediate in its sexual differentiation between B♂ and ♀, entirely agreeing with the morphological one.

3) From the genetical viewpoint, the two patterns of male, B♂ and L♂, were found to transmit from male to male in the successive generation, and so considered as a case of the sex-limited inheritance. Available evidence was corroborated from the two genetical experiments, selection and crossing. Then it was assured that there should be two kinds of the Y chromosome, one of which,  $Y^B$ , bears the strong male-decided factor, and the other,  $Y^L$ , the weak male-decided factor, apparently giving rise to two different patterns for the male plant, B♂ and L♂, respectively.

4) From the breeding viewpoint, it may be noteworthy that the breeding procedure of making an improved race with only one superior pattern of male, L♂, which thus breeds true for L♂, consists either in only one selection or in only one crossing, based on the sex-limited inheritance.

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## CYTOGENETIC STUDIES OF TRISOMICS IN BARLEY<sup>1)</sup>

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### I. INTRODUCTION

Barley is one of the genetically most extensively investigated crop plants. As can be learned from the reports of Robertson, Wiebe and Immer [57], Nagao and Takahashi, M. [46], Robertson, Wiebe and Shands [58, 59], Smith [63], and Takahashi, R. [66, 67], seven possible genetic linkage groups of barley have been already established and linkage maps were to an extent completed by the ordinary method of genetic analysis [80].

The chromosome studies, on the other hand, have recently been advanced by the new aceto-orcein squash technique devised by Tjio and Levan [69]; Karyotypes of normal diploids and some interchange homozygotes were investigated by Hagberg and Tjio [26, 27], Tjio and Hagberg [68] and Tjio and Levan [69] (cf. [14, 23, 24, 80]), and a tester set of translocations were also established by Burnham [12], Burnham *et al.* [15] and Kramer *et al.* [37] (cf. [14]).

Much efforts have been devoted to associate the chromosomes with the respective genetic linkage groups. Some success in this field was achieved using the new excellent cytological techniques [26, 27, 68, 69] and the translocation testers mentioned above [12, 13, 14, 15, 24, 25, 29, 30, 31, 37, 51] (cf. [80]). The most stimulating finding among others is that obtained by Kramer, Veyl and Hanson [37], who indicated that two genetic linkage groups, III and VII, might be associated with only one and the same chromosome and thereby reduced the number of genetic linkage groups of barley to six.

It is generally recognized that trisomics are most useful to identify the chromosomes with their respective linkage groups and also establish the independence of the linkage groups in plants as has been shown in *Zea* [18, 43, 44] and *Lycopersicon* [38, 39, 56]. Nevertheless, the trisomics have not been used as yet in barley for genetic studies owing to the difficulty in inducing the primary simple trisomic types as has been pointed out by Smith [63] and Tsuchiya [82].

The present author was successful in inducing a considerable number of primary simple trisomic plants, comprising all the possible seven types in barley [77, 78, 82, 83]. These trisomics are mostly characterized by normal growth and high seed fertility, which enabled to pursue further the detailed nature of trisomics in barley. In this paper are described the results of cytogenetic studies and establishment of all possible seven types of primary simple trisomics from autotriploids in barley. The further purpose of this paper is to standardize the primary simple trisomic types and to determine the relations between the extra chromosomes and the genetic linkage groups of barley.

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## II. ORIGIN AND OCCURRENCE OF TRISOMIC PLANTS

The original material used in this study is the diploid species of a wild two-rowed barley, *Hordeum spontaneum* C. Koch var. *transcaspicum* Vav. having  $2n=14$  chromosomes. Autotriploid plants were obtained by the crossing between induced autotetraploids and the normal diploids of this species [75, 79], though the production of triploids seemed to be very difficult in barley [28, 63, 72, 73, 79]. The self-pollinated autotriploids and crosses of the autotriploids with diploids gave many trisomics and some other chromosomal types [77, 82].

Among various chromosomal types which appeared in the progenies of autotriploid, simple trisomic plants with  $2n=15$  chromosomes were most frequent, though their frequency differed with method of breeding and varied also from year to year. Out of 96 plants in the progenies of selfed autotriploids 43 (44.8%) were simple trisomics; diploids amounted to 20.8 percent and double trisomics to 19.8 percent. In the progenies of  $3x \times 2x$ , simple trisomics occurred most frequently (56.7% of all 30 plants) which was followed by diploid type (40%). Among the progenies of the triploids, selfed  $3x$  and  $3x \times 2x$ , which amounted 126 plants 59 (46.6%) were simple trisomics. Besides these, double ( $2n=16$ ), triple ( $2n=17$ ), and presumably quadruple trisomics ( $2n=18$ ), true triploid ( $2n=21$ ), hyper-triploid ( $2n=25$ ) plants, the diploid and the simple trisomic plants with an additional fragment ( $2n=14+1f$ ,  $15+1f$ ) occurred in various frequencies (Table 1). The frequency of occurrence of simple trisomic plants and other chromosomal types in progenies of autotriploids is presented in Table 1 [82].

In 1953 twenty four simple trisomics were found together with other chromosomal types among the progenies of four autotriploids of *H. spontaneum* var. *transcaspicum* (Tables 1, 2). Chromosome configurations of 19 trisomics and the progenies of all 24 trisomics, at diakinesis and/or MI were investigated which showed the primary nature of the extra chromosome (Table 5; cf. [2, 3]). The 24 simple trisomics were classified into 7 types mainly by their gross morphological traits and some preliminary measurements of several characters [82]. They were tentatively named as: Bush, Slender, Pale, Robust, Pseudo-normal<sup>1)</sup>, Purple and Semi-erect [77, 82].

In the following year (1954) thirty simple trisomics were obtained from 9 autotriploid plants of the same species (Tables 1, 2). All these simple trisomics again were classified into seven different groups which corresponded to those mentioned above (Table 2). Further in 1954 many simple trisomics were obtained in the progenies of double and triple trisomics which were derived from autotriploids in 1953 as siblings of simple trisomics just mentioned (Table 1). They also were classified into seven groups by their resemblance with the previous types (Table 2). Thus, in this case all simple trisomics were obtained from autotriploids in their direct progeny or indirectly through double and triple trisomics (Tables 1, 2).

1) This was first named "Normal" (Tsuchiya 1954[77]) which was later changed to "Pseudo-normal" upon Dr. Kihara's suggestion.

TABLE 1

Chromosome constitution of offspring obtained from autotriploid wild barley,  
*Hordeum spontaneum* var. *transcaspicum* selfed and crossed with diploids

Chromosome number (2n)	3x selfed			3x × 2x			Grand total
	1953	1954	Total	1953	1954	Total	
14	15 (28.8) %	5 ( 11.4) %	20 (20.8) %	4 ( 40.0) %	5 ( 25.0) %	9 (30.0) %	29 ( 23.0) %
14+1f*	1 ( 1.9)	1 ( 2.3)	2 ( 2.1)	0	0	0	2 ( 1.6)
15	19 (36.5)	24 ( 54.5)	43 (44.8)	5 ( 50.0)	11 ( 55.0)	16 (53.3)	59 ( 46.7)
15+1f*	1 ( 1.9)	2 ( 4.5)	3 ( 3.1)	0	0	0	3 ( 2.4)
16	10 (19.2)	9 ( 20.5)	19 (19.8)	1 ( 10.0)	2 ( 10.0)	3 (10.0)	22 ( 17.5)
17	3 ( 5.8)	2 ( 4.5)	5 ( 5.2)	0	1 ( 5.0)	1 ( 3.3)	6 ( 4.8)
18	0	1 ( 2.3)	1 ( 1.0)	0	0	0	1 ( 0.8)
19	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0
21	1 ( 1.9)	0	1 ( 1.0)	0	0	0	1 ( 0.8)
25	1 ( 1.9)	0	1 ( 1.0)	0	0	0	1 ( 0.8)
Not identified	1 ( 1.9)	0	1 ( 1.0)	0	1 ( 5.0)	1 ( 3.3)	2 ( 1.6)
Total	52 (99.8)	44 (100.0)	96 (99.8)	10 (100.0)	20 (100.0)	30 (99.9)	126 (100.0)

\* Fragment.

TABLE 2

Frequency of seven types of primary simple trisomics in the progenies of  
autotriploids and double or triple trisomic plants of barley

Type of trisomics	From autotriploid progeny			From double and triple trisomics	Grand total
	1953	1954	Total		
Bush	5 (20.8) %	3 ( 8.6) %	8 (13.6) %	1 ( 5.6) %	9 (11.7) %
Slender	3 (12.5)	8 (22.9)	11 (18.6)	3 (16.7)	14 (18.2)
Pale	6 (25.0)	10 (28.6)	16 (27.2)	3 (16.7)	19 (24.7)
Robust	2 ( 8.3)	2 ( 5.7)	4 ( 6.8)	3 (16.7)	7 ( 9.1)
Pseudo-normal	1 ( 4.2)	3 ( 8.6)	4 ( 6.8)	3 (16.7)	7 ( 9.1)
Purple	3 (12.5)	6 (17.2)	9 (15.3)	3 (16.7)	12 (15.6)
Semi-erect	4 (16.6)	3 ( 8.6)	7 (11.8)	2 (11.1)	9 (11.7)

The frequency of each trisomic type in the progeny of autotriploids varies from type to type. In the progenies of selfed autotriploids, Robust did not appear in 1953 neither did Bush in 1954. Pale occurred most frequently in both years. On the whole, Pale amounted to 34.9 percent of all trisomics obtained from selfed autotriploids. In the progenies, of 3x × 2x, Slender was found most frequently (31.3%), being followed by Bush (25.0%). Robust and Pseudo-normal appeared only 4 each (6.8%) among all 59 simple trisomics, while Pale amounted to 27.2 percent

TABLE 3  
Diagnostic morphological features of the seven primary simple trisomics in barley as expressed under field condition

Types of trisomics	Growth habit	Leaf characters*	Other outstanding features	Corresponding type of	
				Ramage (1955)	Tsuchiya (1952a)
Bush	Bushy; dwarf with many tillers.	Short, narrow, dark green color; frequent occurrence of fused leaves. Index 20.0.	Short ears with relatively long awns; multiple or compound spikelets in 26% of spikelet triplets. Degeneration of one or two anthers in 34% of florets. Very long empty glumes. Rachilla frequently changes to awn-like body.	b	(T20 - 9)**
Slender	Bushy with many tillers; slender appearance in general aspects.	Very long, thin, narrow and hanging down at almost vertical position; dark green. Index largest, 25.3.	Slenderness in all the plant parts; short awn; very short stomatal guard cells; small seeds. Relatively low seed fertility.	f	T20 - 12
Pale	Nearly normal with slightly oblique stems; seemingly weak.	Extremely twisted leaf tips with pale color; thin and drooping at almost vertical position; very small flag leaf. Index 17.7.	Dense ears with short and weak awns. Short empty glumes. Tiny rachilla. Emaciated seeds. Low pollen fertility.	c	(T20 - 8)
Robust	Robust and vigorous.	Long, wide, thick and dark green with very wavy margins. Index 13.3.	Long ears with long and coarse awns. Thin and soft glumes. Emaciated seeds.	e	(T20 - 11)
Pseudo-normal	Closely resembles normal diploids.	Inversely twisted small leaves. Index 16.6.	Plants nearly normal appearance but somewhat smaller than diploids. Small ears with short and thin awns. Empty glumes and rachilla are short. Seeds small.	a	—
Purple	Robust and coarse, semi-prostrate.	Thick, wide, long, coarse and dark green; dark purple color in leaf sheaths. Index 14.0.	Shorter ears with relatively long and coarse awns. Extremely long empty glumes. Rachilla axis is long but rachilla hairs very short. Seeds shorter and wider.	g	T20 - 7
Semi-erect	Semi-erect with short and straight leaves.	Straight, short, and wide leaves with coarse texture. Index smallest, 12.8.	Short and lax ears with short and coarse awns. Large seeds. Very large empty glumes. Rachilla axis and rachilla hairs are the longest of all seven types.	d	(T20 - 10)
Diploid	Prostrate or spreading type.	Relatively long with slightly waved margins. Index 16.4.	See Table 10		

\* Leaf index (length/width ratio) was calculated from a measure of the leaf preceding the flag leaf.

\*\* The plants presented in parentheses presumably correspond to respective trisomic type.



of all the simple trisomics obtained from the progenies of triploids (Table 2). Primary simple trisomics obtained from double and triple trisomics were classified into seven types and the frequency differed with different types (Table 2).

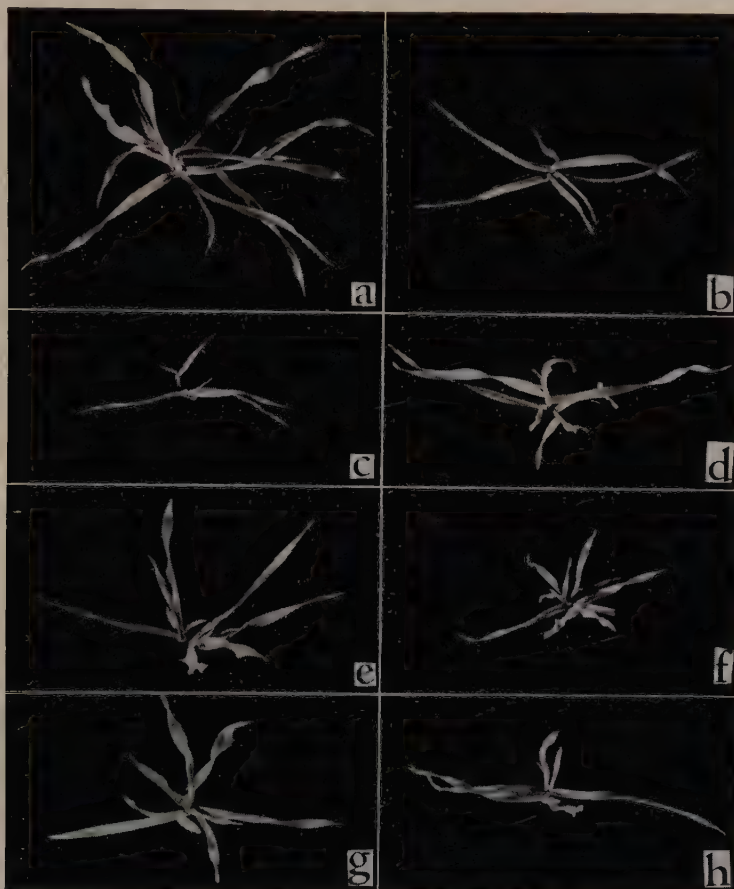


Fig. 1. Seedlings of diploid (a) and trisomics (b~h) viewed from above.  $\times 0.66$ .  
a, diploid. b, Bush. c, Slender. d, Pale. e, Robust. f, Pseudo-normal.  
g, Purple. h, Semi-erect.

Thus the frequency of occurrence of the primary simple trisomic types is shown to vary from year to year and also with different sources (Tables 1, 2, cf. [101]). The same situation has been frequently found in *Datura* [7, 8, 10], tomato [38, 39, 56], *Nicotiana sylvestris* [21, 22], maize [43, 44] and others.

### III. CHARACTERISTICS OF PRIMARY SIMPLE TRISOMICS

The primary simple trisomics herein reported have been obtained from a diploid wild species, *H. spontaneum* C. Koch var. *transcaspicum* Vav. The diploid plants have many wild characters which breed true and seemed to be stable.

Secondry, all the trisomics were derived from autotriploid plants (cf. [75, 79]). The autotriploids showed to have only a few chromosomal and/or genic changes ([75], Tsuchiya unpublished) as compared with other sources.



Fig. 2. Leaves of trisomic (upper row) and diploid (lower row) seedlings at early stage.  $\times 0.6$ .

Upper row: Trisomics from trisomic parents.

Lower row: Diploids from diploid (a) and trisomic parents of Bush (b), Slender (c), Pale (d), Robust (e), Pseudo-normal (f), Purple (g), and Semi-erect (h).

mentioned above become clear at later growing stages especially in the tillering stage but they are to some extent recognizable at earlier stages. Robust have slender first foliage leaf with blue green color and Purple have only slightly twisted first foliage leaf (Fig. 2 e, g). Culms, leaves, spikes, seeds and some other characters were measured and are comparatively represented in the previous paper [82] and some are illustrated in Figs. 1~5.

#### IV. FERTILITY AND GERMINATION CAPACITY

Many of the barley trisomics previously reported had low fertility [35, 50, 57, 71, 72], especially those obtained by Ramage [50] from translocation heterozygotes; all seven types were highly or almost completely sterile (cf. [82]). Some

Each of the primary simple trisomic types differs from the normal diploid of *H. spontaneum* var. *transcaspicum* in a large number of characters; some differences are minute and inconspicuous, others gross and striking; some are cytological, morphological or physiological nature; and some are quantitative or qualitative. Each trisomic type represents a unique combination of a number of characters (Figs. 1~5).

Since a detailed descriptions and illustrations have been given in previous papers [77, 82, 83], the names and some brief accounts will be given in Table 3.

As has been reported in previous papers [77, 82, 83] and presented in Table 3 of this paper, five of the 7 primary simple trisomic types are at early seedling stage readily distinguishable from each other as well as from normal diploids, even in varied genetic backgrounds. In the remaining two types, Robust and Purple, their robust, coarse nature and other distinctive traits men-



Fig. 3. Plants of diploid and trisomics at the stage preceding the heading.  $\times 0.4$ . a, diploid. b, Bush. c, Slender. d, Pale. e, Robust. f, Pseudo-normal. g, Purple. h, Semi-erect.

trisomics obtained by the present author from a hypotriploid plant of Early Golden Melon showed also complete or high sterility when selfed [71, 72]. On the contrary, the trisomics here obtained had fairly good fertility (Table 4).

#### 1. *Pollen fertility*

Pollen fertility of barley trisomics has not been previously reported with the exception of the present author's report. In 4 trisomic plants examined it was shown to be relatively high in fertility ranging from 76 to 96 percent [71, 72].





Fig. 4. Spikes of diploid and trisomics.  $\times 0.48$ .

a, diploid. b, Bush. c, Slender. d, Pale. e, Robust. f, Pseudo-normal. g, Purple. h, Semi-erect.



Fig. 5. Comparison of seed size of trisomics (upper row) and diploids (lower row).  $\times 2.4$ .

Upper row: The seeds from which trisomics emerged.

Lower row: The seeds from which diploids emerged.

These seeds produced by parental diploid (a), Bush (b), Slender (c), Pale (d), Robust (e), Pseudo-normal (f), Purple (g), and Semi-erect (h).

Pollen fertility of the seven trisomic types is very high with the exception of Pale. The six trisomics had more than 90 percent of good pollen grains (Table 4, cf. [101]). In Pale, however, the pollen fertility was 72.3 percent; about 30

percent of the pollen grains contained one or two degenerated nuclei.

The pollen grains of the seven trisomics are represented in previous paper [82]. It has been shown that the pollen grains of each trisomic might be divided into two groups by their size. The same situation was observed by Nishiyama in a trisomic *Avena* plant [48]. The size of pollen grains seems to be smaller in Slender and Pseudo-normal than in the other 5 trisomics and the diploids [82].

## 2. Seed fertility

The seed fertility of the seven trisomic types was relatively high in comparison with other barley trisomics previously reported [50, 62, 72]. However, there were observed some differences in different trisomic types, different years, and different methods of breeding (Table 4). It is noteworthy that in all trisomic types completely fertile spikes were sometimes observed.

Self fertility in bagged condition varied from 65.1 percent in Slender to 90.5 percent in Pseudo-normal, the average of seven types being 80.2 percent. Seed fertility in open-pollination is closely similar to that of selfed ears; the average of seven types was 83.6 percent (Table 4). The cause of the lowest fertility in Slender

TABLE 4  
Fertility and germination percentage of seven primary simple trisomics

Types of trisomics	Pollen fertility	Seed fertility				Germination percentage		
		Selfing	Open	$(2x+1) \times 2x$	$2x \times (2x+1)$	Selfing	$(2x+1) \times 2x$	$2x \times (2x+1)$
Bush	%	%	%	%	%	%	%	%
Bush	97.2	68.0	72.1	82.5	91.4	90.3	85.3	84.4
Slender	94.7	65.1	73.2	71.1	93.7	76.8	76.5	81.8
Pale	72.3	88.3	88.5	81.3	97.9	81.4	85.5	73.4
Robust	92.6	80.5	90.3	87.3	96.1	95.0	89.3	85.7
Pseudo-normal	93.6	90.5	91.0	76.6	94.7	86.4	86.4	72.0
Purple	96.5	82.8	89.2	82.0	97.2	90.8	89.9	61.5
Semi-erect	96.6	86.2	81.1	81.7	91.7	83.9	86.3	71.3
Average	91.9	80.2	83.6	80.4	95.2	86.4	85.6	75.2
Diploid	99.2	91.3	97.2	—	—	93.2	—	—

is unknown, but the extremely reduced seed fertility in Bush may be partly ascribed to the frequent occurrence of multiple spikelets which were usually sterile.

The fertility of  $(2x+1) \times 2x$  was closely similar to that of selfings and open-pollination; the average was 80.4 percent ranging from 71.1 percent in Slender to 87.3 percent in Robust. In this case the fertility of Bush was more than 10 percent higher than in selfing (Table 4). This increase in seed fertility of  $(2x+1) \times 2x$  in Bush compared with selfing may be explained by the fact that in this case only the normal florets were used in the crosses in contrast to selfing where the sterile multiple spikelets were included in fertility calculation.

The highest seed fertility was observed in the cross  $2x \times (2x+1)$ . All the seven types showed very high fertility ranging from 91.4 to 97.9 percent, the average of the seven types being 95.2 percent.

On an average for selfings, open-pollinations and  $(2x+1) \times 2x$ -cross, the lowest fertility is observed in Slender (69.8%) which is followed by Bush (74.2%). The remaining 5 types showed fertility closely similar to each other; Pale, Robust, Pseudo-normal, Purple and Semi-erect showed 86.0, 86.0, 85.8, 84.7, and 83.0 percent, respectively.

The trisomic plants previously reported by the present author showed an extreme variation of seed fertility ranging from complete sterility to almost complete fertility in selfing [72]. A similar situation of wide variation in seed fertility was observed in auto- as well as allotrisomic plants derived from triploid *Avena* hybrids; some were highly or completely sterile and others had very high seed fertility [48]. Einset [17] reported almost complete seed fertility in eight of the possible ten primary simple trisomic types of maize; the average of eight types was 92~98 percent ranging from 75~100 percent.

### 3. Germination percentage

Germination percentage was studied in seeds obtained from selfings of trisomics,  $(2x+1) \times 2x$  and  $2x \times (2x+1)$ . The germination was very good in all cases. In selfed seeds the germination percentage varied from 76.8 percent in Slender to 95.0 percent in Robust, the average being 86.4 percent. The germination percentage of seeds obtained from the cross  $(2x+1) \times 2x$  ranged from 76.5 percent in Slender to 89.9 percent in Purple, the average of 7 types being 85.6 percent. Thus, in these two cases, the lowest germination was observed in Slender which also showed the lowest seed fertility (Table 4).

The germination percentage of  $2x \times (2x+1)$  was highest in 1957 [82]. In 1958, however, it was very low. The average values from two years are presented in the last column of Table 4. The average of the seven types was 74.3 percent ranging from 59.6 percent in Purple to 83.2 percent in Robust.

## V. CYTOLOGICAL STUDIES OF TRISOMIC TYPES

The primary simple trisomics are classified into seven different types mainly by their external morphology [77, 82, 83]. Identification of the seven types on karyomorphological basis was attempted. The karyotype analysis of the trisomics herein described is, however, restricted to 3 or 4 types owing to the difficulty in distinguishing the largest 3 or 4 chromosomes in somatic metaphase plates (cf. [68]). Pachytene analysis may not be applied for the identification of the extra chromosomes in barley trisomics at least at present technical level (cf. [60]). However, the study of meiotic chromosome behaviors gives some informations on the independence of the seven trisomic types (Tables 5, 6; Figs. 7~13). Further, a detailed study of diplotene and diakinesis furnished some evidences as to the nucleolus-chromosome relationships in barley [84] (cf. [74, 80]) which also support to some extent the independence of the trisomic types on the cytological basis.



### 1. *Karyotype analysis*

Karyomorphology in diploid barley has recently become advanced [14, 23, 25, 26, 27, 49, 68, 80] since the introduction of a new acetic orcein squash technique by Tjio and Levan [69]. However, it has been shown to be difficult to distinguish the largest 3 or 4 chromosomes which are closely similar as to the length of chromosomes and the position of kinetochore [14, 23, 25, 26, 27, 68, 69, 80].

It seems to be more difficult to determine these 3 or 4 longest chromosomes as the extra chromosome in each of the trisomic types. Identification of the SAT-chromosomes was successful in two trisomic types, Purple and Semi-erect, which have each one of the two SAT-chromosomes in triplicate condition. The karyotype of Pseudo-normal is almost ready; it has been shown to have the shortest chromosome extra.

In Fig. 6 somatic metaphase plates of the diploid (a) and the seven trisomics (b~h) are represented. As clearly shown in Fig. 6 g, the extra chromosome of Purple is chromosome 6 (Burnham's *g*) with the large satellite. This finding has been verified by the study of nucleolus-chromosome relationships at diakinesis and late diplotene stage of meiosis (Fig. 12).

Another trisomic type, Semi-erect, in which the karyotype analysis was completed showed to have the chromosome 7 (Burnham's *d*) with the small satellite in triplicate condition. The secondary constriction in this trisomics only slightly exhibited in somatic metaphase plates (Fig. 6 h).

These two cases are very clear-cut owing to the distinctive morphology of the extra chromosomes having satellites. Therefore, no measurements of the somatic chromosomes have been made. In the other 5 types preliminary measurements were made to identify the extra chromosomes by the point diagram analysis [68]. As a result the extra chromosome of Pseudo-normal was shown to be chromosome 5, the shortest one of the barley chromosome complement (Burnham's *a*; Fig. 6 f). This finding confirmed that the linkage group II was carried by the extra chromosome of Pseudo-normal and also by Burnham's *a* chromosome which was determined to be the smallest one [14, 25, 27, 37, 82].

Measurements of somatic chromosomes made in the metaphase plates of Robust (Fig. 6) did not give a conclusive evidence as to the shape of the extra chromosome. The somatic chromosomes of the remaining 3 trisomic types, Bush, Slender, and Pale, are presented in Figs. 6 b~d. The study of these plates did not allow an accurate identification of the extra chromosomes as to their shape. The conclusions must be postponed until a further extensive study is made.

### 2. *Meiosis in microsporocytes of homozygous trisomics*

Chromosome behaviors at earlier stages than late diplotene or early diakinesis were not studied in detail. At diplotene and diakinesis the association of chromosomes, the types of trivalents (Table 5) and the nucleolus-chromosome relationships were observed (Table 6). At MI the association of chromosomes, types of trivalents were observed in some detail (Table 5).

At late diplotene and early diakinesis in the microsporocytes of all trisomic plants the chromosome association of  $1_{III}+6_{II}$  is prevalent and  $7_{II}+1_I$  less fre-

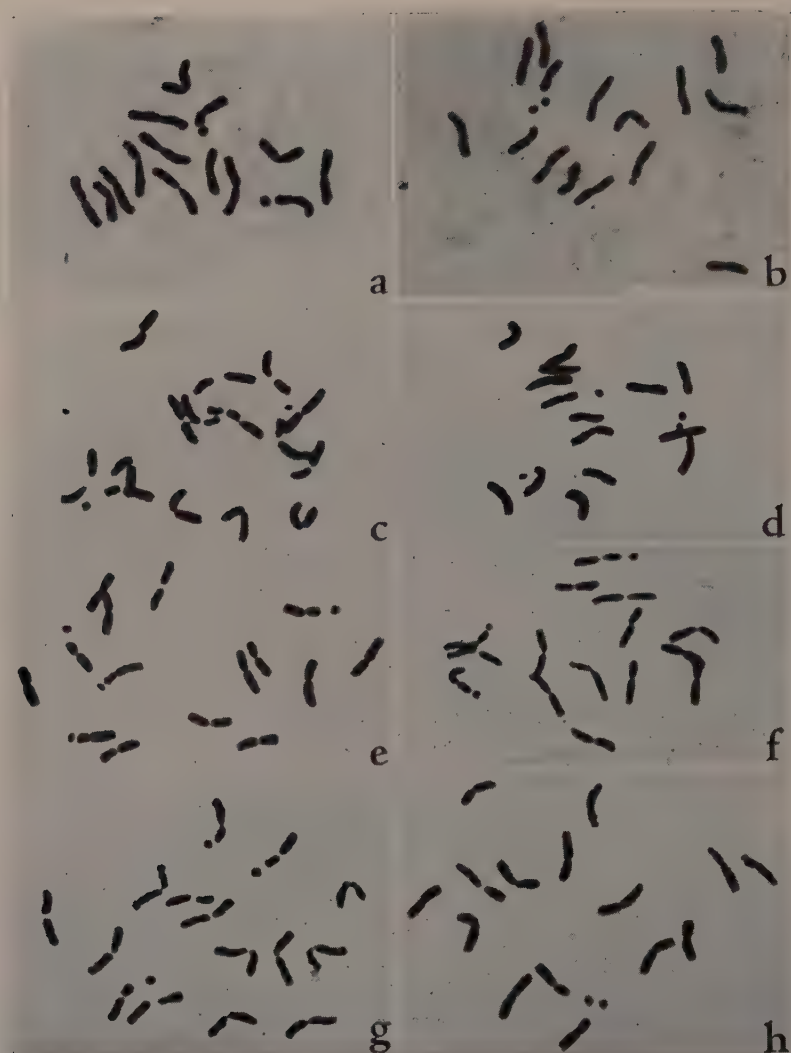


Fig. 6. Somatic metaphase chromosomes in root tip squashes of diploid ( $2n=14$ ) and trisomics ( $2n=15$ ) of barley.  $\times 900$ .

a, diploid. b, Bush. c, Slender. d, Pale. e, Robust. f, Pseudo-normal. g, Purple; showing three chromosome 6 with larger satellite. h, Semi-erect; showing three chromosome 7 with smaller satellite.

quent (Table 5. cf. [72]). Other configurations with failure of pairing in Slender (Fig. 8d, g; cf. [85]) and multiploid sporocytes in Pale (Fig. 9g~j; cf. [85]) were observed occasionally.

In almost all of the sporocytes one nucleolus occurred but in some cases two or more nucleoli were observed with varied frequency in the different trisomic types (Table 6). In many sporocytes of 6 trisomic types except for Purple

TABLE 5  
Chromosome associations and types of trivalents at diakinesis (DK) and metaphase-I (MI) in seven trisomic types (in percent)

Types of trisomics	Stage	Chromosome associations				Types of trivalents			
		1III+6II	7II+1I	Others	No. spor. obsvd.	Chain	Frying-pan	Y-type	Triple-arc
Bush	DK	82.9	17.1	—	140	27.6	60.3	0	12.1
	MI	70.7	28.7	0.7	150	67.3	31.8	0.9	0
Slender	DK	89.3	10.7	—	420	53.1	43.2	1.1	2.7
	MI	78.6	20.0	1.4	1,280	69.2	29.8	0.4	0.6
Pale	DK	94.3	5.7	—	265	40.8	56.0	0	1.0
	MI	76.0	23.8	0.2	2,060	62.4	36.1	0.9	0.6
Robust	DK	92.9	7.1	—	42	46.2	48.7	0	5.1
	MI	77.7	21.9	0.4	480	64.9	33.0	1.1	1.1
Pseudo-normal	DK	79.5	20.5	—	200	49.7	47.1	0.6	2.5
	MI	63.1	36.5	0.4	260	72.0	25.6	1.8	0.6
Purple	DK	89.0	11.0	—	100	41.6	53.9	0	4.5
	MI	77.7	22.3	0.1	1,200	60.2	37.8	0.5	0.1
Semi-erect	DK	93.9	6.1	—	262	20.3	70.7	0.8	8.1
	MI	71.7	28.2	0.2	660	47.1	46.7	5.7	0.4
Average	DK	89.1	10.9	—	1,429 (Total)	40.6	54.1	0.4	4.9
	MI	75.9	23.7	0.5	6,090 (Total)	62.5	35.5	1.3	0.02



TABLE 6  
Frequency of sporocytes with varying number of nucleolus at late prophase of  
meiosis in trisomic plants (in percent)

Types of trisomics	Number of nucleolus per sporocyte				Number of sporocytes observed
	1	2	3	4	
Bush	97.9	2.1	0	0	140
Slender	98.3	1.7	0	0	420
Pale	98.1	1.9	0	0	265
Robust	100.0	0	0	0	42
Pseudo-normal	98.5	1.5	0	0	200
Purple	88.0	10.0	1.0	1.0	100
Semi-erect	93.9	5.7	0.4	0	262
<i>Average</i>	<i>96.9</i>	<i>2.9</i>	<i>0.1</i>	<i>0.1</i>	<i>1,429</i> (Total)
Diploid	99.2	0.8	0	0	509

one or two bivalents touched the nucleolus or nucleoli (Figs. 7~11, 13). However, in some sporocytes of Semi-erect trisomic for chromosome 7 with the smaller satellite the trivalent touched the nucleolus together with one bivalent (Fig. 13 a~d), indicating that the chromosome 7 is obviously one of the nucleolus organizing chromosomes [15, 32, 73, 74, 80, 84]. In Purple trisomic for chromosome 6

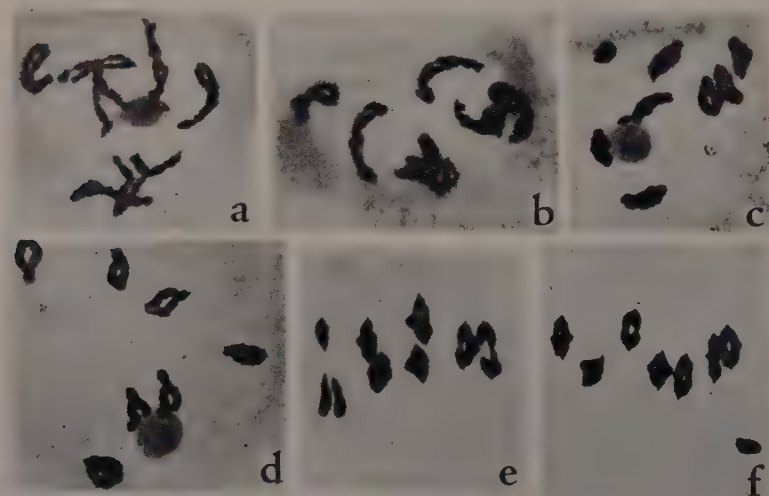


Fig. 7. Meiosis in Bush.  $\times 1,000$ . a~d, diakinesis. a,  $1_{III}+6_{II}$ ; two bivalents touched the nucleus with large and small satellite, respectively. b,  $1_{III}+6_{II}$ ; two nucleoli each of which attached to one bivalent. c,  $7_{II}+1_I$ ; two bivalents attached to the nucleolus. d,  $1_{III}+6_{II}$ ; showing two bivalents attached to the nucleolus, and triple-arc trivalent at seven o'clock. e and f, MI. e,  $1_{III}+6_{II}$ , showing V-shaped trivalent. f,  $7_{II}+1_I$ .

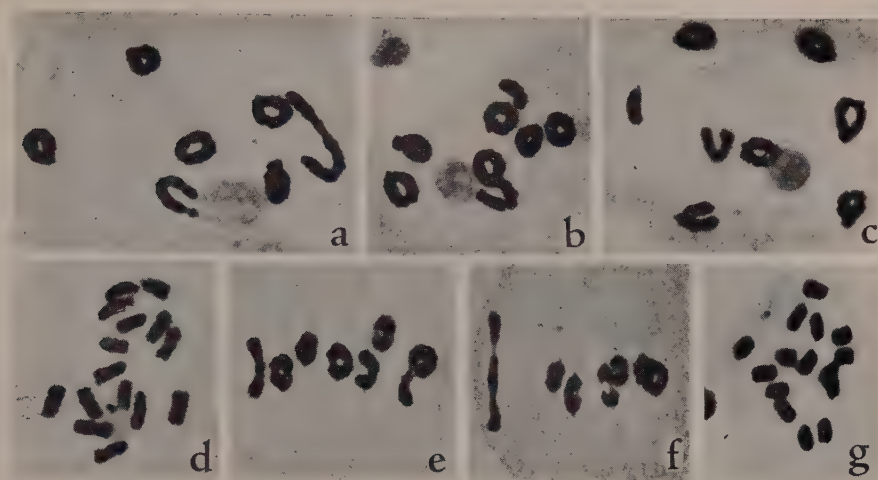


Fig. 8. Meiosis in Slender.  $\times 1,000$ . a~d, diakinesis. a,  $1_{III}+6_{II}$ ; showing a chain trivalent and two bivalents associated with the nucleolus by large and small satellite, respectively. b,  $1_{III}+6_{II}$ ; showing the frying-pan trivalent and a lemon-shaped nucleolus attached by 2 bivalents from opposite side. c,  $7_{II}+1_{I}$ ; showing one bivalent touched the nucleolus. d, asynaptic sporocyte with  $15_{I}$ ; showing 4 or 5 univalents associated the nucleolus. e~g, MI. e~f,  $1_{III}+6_{II}$  with frying-pan (e) and rod-type trivalent (f). g, asynaptic sporocyte with  $15_{I}$ .

(Burnham's *g*) with the large satellite, all but a few sporocytes showed the trivalent attached to the nucleolus (Fig. 13; cf. [80, 84]).

At late diplotene and diakinesis of some sporocytes in 4 trisomic types, Bush, Slender, Pale and Pseudo-normal, the trivalent or a univalent touched the nucleolus together with other bivalents indicating that the extra chromosome of those trisomics have a weak capacity of nucleolus organization.

The shape of trivalents at diakinesis was frying-pan, chain, Y, or triple-arc; the first was most frequent and its average frequency of the seven trisomic types being 54.1 percent (43~70%) which was followed by the second with the frequency of 40.6 percent (20~53%), the last two being very few (Table 5).

At MI various types of chromosome associations and several types of trivalents were observed (Table 5; Figs. 7~13). In all trisomic types the frequency of chromosome association of  $1_{III}+6_{II}$  was reduced at MI and the  $7_{II}+1_{I}$  correspondingly increased; the average of the seven types is 75.9 percent for the former and 23.7 percent for the latter association (Table 5), while the corresponding values at diakinesis are 89.1 percent and 10.9 percent, respectively (Table 5; cf. [72]). The types of trivalents at MI also differ in their frequency from those at diakinesis; the chain increases and the frying-pan and other complicated types are significantly reduced, the average of seven types being 44.8 percent for chain, 35.5 percent for frying-pan and only 2.1 percent for the others (Table 5; cf. [72]).

At AI the chromosomes separated to 7~8 in many sporocytes, while one lagging chromosome is observed frequently. The dividing halves of a lagging

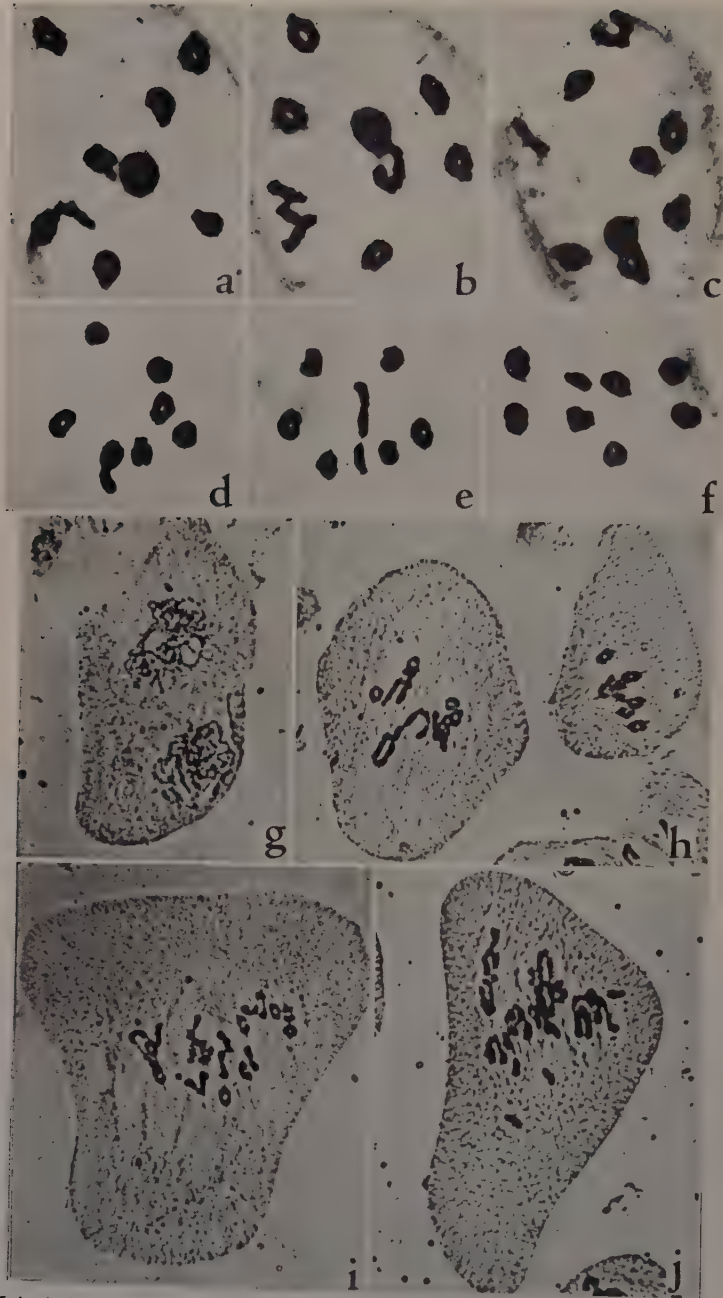


Fig. 9. Meiosis in *Pale*. a~f,  $\times 1,000$ . g~j,  $\times 530$ . a~c, diakinesis. a and b,  $1_{III}+6_{II}$  showing frying-pan (a) and chain (b) trivalent; in both sporocytes one bivalent attached to the nucleolus. c,  $7_{II}+1_I$ , one bivalent associated with the nucleolus. d~f, MI. d and e,  $1_{III}+6_{II}$  showing frying-pan (d) and rod-type trivalent (e). f,  $7_{II}+1_I$ . g~j, Syncyte formation in *Pale*-2-6 (g~i) and *Pale*-4-2 (j). g, a syncyte with two pachytene nuclei in a plasmodium. h, two sporocytes at MI; one syncyte consisted of two nuclei ( $1_{VI}+2_{IV}+8_{II}$ ) and another normal cell showing  $1_{III}+6_{II}$ . i, a syncyte consisted of 3 nuclei with the configuration of  $1_V+1_{III}+18_{II}+1_I$ . j, a syncyte at MI consisted of 4 nuclei with many quadrivalents, trivalents and univalents.

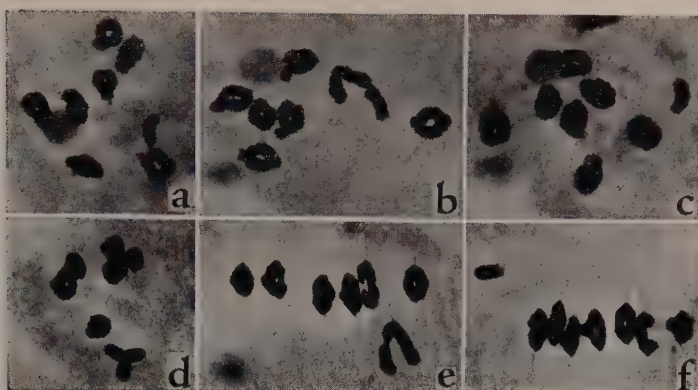


Fig. 10. Meiosis in Robust.  $\times 990$ . a~c, diakinesis. a,  $1_{III}+6_{II}$  with ring-and-rod trivalent; two bivalents touched the nucleolus. b,  $1_{III}+6_{II}$  with chain trivalent; the nucleolus associated with one bivalent. c,  $7_{II}+1_I$ ; two bivalents associated with the nucleolus. d~f, MI. d and e,  $1_{III}+6_{II}$  showing Y-shaped (d) and V-shaped trivalent. f,  $7_{II}+1_I$ .

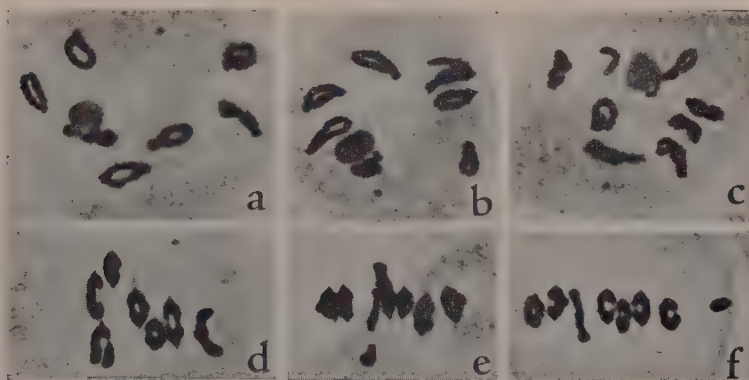


Fig. 11. Meiosis in Pseudo-normal.  $\times 1,000$ . a~c, diakinesis. a and b,  $1_{III}+6_{II}$  showing triple-arc at 2 o'clock (a) and ring-and-rod trivalent (b); one bivalent attached to the nucleolus. c,  $7_{II}+1_I$ ; one bivalent attached to the nucleolus. d~f, MI. d and e,  $1_{III}+6_{II}$  showing V- (d) and rod-shaped trivalent (e). f,  $7_{II}+1_I$ .

univalent usually passed to the poles to be included in the daughter nuclei. At interkinesis almost all sporocytes have normal appearance without micronuclei but there are some with one or two micronuclei.

The second metaphase plates show usually regular arrangement of chromosomes; less frequently irregular plates were observed. At AII 8-8 and 7-7 distributions were most frequently met with. The figure 7-1-7 was also observed occasionally. The laggards at AII and TII especially in the latter showed sometimes clearly their shape (cf. [72]) as in a rye trisomic [65]. Morrison [45] identified the types of monosomics of hexaploid wheats by the study of the shape of such AII~TII laggards.



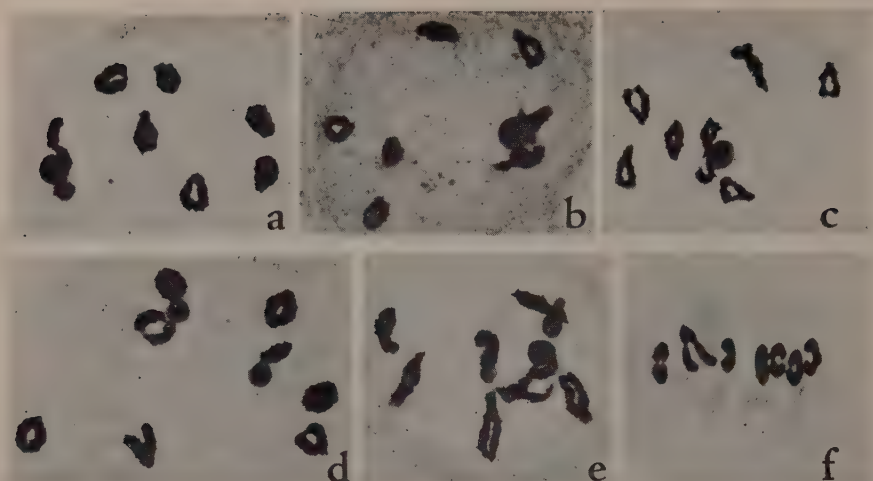


Fig. 12. Meiosis in Purple.  $\times 1,000$ . a~e, diakinesis. a,  $1_{III}+6_{II}$  showing the chain trivalent touched the nucleolus. b,  $1_{III}+6_{II}$  showing the nucleolus attached to the trivalent and a bivalent. c,  $1_{III}+6_{II}$  showing the two nucleoli each of which associated with trivalent and a bivalent, respectively. d,  $7_{II}+1_I$  showing two nucleoli each of which associated with two bivalents and one univalent. e,  $1_{III}+6_{II}$  showing 4 nucleoli of which the largest was attached by the chain trivalent, the second and the smallest associated with a bivalent and the 3rd sized with still other bivalent. f,  $1_{III}+6_{II}$  at MI showing V-shaped trivalent.

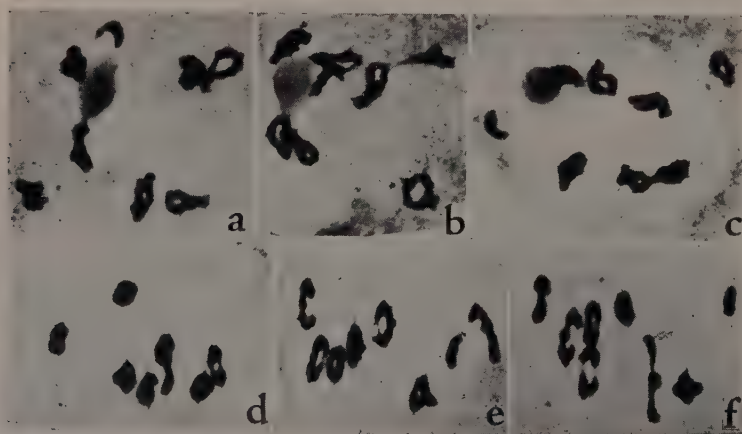


Fig. 13. Meiosis in Semi-erect.  $\times 1,000$ . a~c, diakinesis. a and b,  $1_{III}+6_{II}$  showing the lemon-shaped nucleolus which was attached by the trivalent and a bivalent from opposite side. c,  $7_{II}+1_I$ ; one bivalent associated with the nucleolus. d~f, MI. d and e,  $1_{III}+6_{II}$  showing ring-and-rod (d) and V-shaped trivalent (e). f,  $7_{II}+1_I$ .

At quartet stage majority of the sporocytes form normal tetrads without micronuclei, but tetrads with micronuclei are also found, though they were less frequent. The microspores developed well in many cases which results in high

pollen fertility (Table 4).

The chromosome behavior in each trisomic type with special reference to the nucleolus-chromosome relationships will be reported in other paper (cf. [84]).

## VI. TRANSMISSION OF THE EXTRA CHROMOSOMES IN TRISOMICS

### 1. *Transmission through egg and pollen*

Among the selfed progeny of homozygous trisomics the lowest transmission was observed in Slender (21.2%) and the highest in Bush (31.4%), the average of the seven types being 25.9 percent. In the hybrid progeny the transmission rates are almost the same as those in the homozygous trisomics with the average of 29.1 percent. In this case also the lowest is Slender (18.6%). The rates of transmission were presented in Table 7.

Transmission rates in barley trisomics have been thoroughly investigated by Ramage [50]. The results here obtained are closely similar to that obtained by Ramage. Ramage also found that no significant differences were observed in the rates of transmission; between trisomic seeds produced in central and lateral florets; between top half and bottom half florets; and also between plants with different genetic constitutions [50].

The egg transmission was investigated in the progenies from the cross  $(2x+1) \times 2x$ . The lowest is 9.8 percent in Pseudo-normal and the highest is 27.5 percent in Slender, the average of the seven types being 22.7 percent, which value is somewhat lower than the average of the selfed trisomics (Table 7).

TABLE 7  
Rates of transmission in trisomic barley

Types of trisomics	Frequency of trisomics in progenies of		
	Selfing	$(2x+1) \times 2x$	$2x \times (2x+1)$
Bush	31.4	24.9	0
Slender	21.2	27.5	0
Pale	21.0	27.2	0
Robust	28.8	11.3	0
Pseudo-normal	24.6	9.8	0
Purple	19.3	17.4	0
Semi-erect	23.4	24.5	0
<i>Average</i>	<i>25.9</i>	<i>22.7</i>	0

The extra chromosomes are not transmitted through the pollen in all the seven trisomic types here investigated (Table 7). In some other plant species, transmission through the pollen was observed [10, 21, 38, 39, 64], though the transmission rates varied extremely from type to type. In *Datura* only 4

among the possible 12 primary types transmitted the extra chromosome through the pollen [10].

The data presented in Table 7 and the results described above indicate that the rates of transmission seem to differ with different types and the method of breeding. However, the transmission rates may be said to be sufficiently high in all the seven types to maintain the stocks by selfings or crosses  $(2x+1) \times 2x$ .

## 2. Transmission rates in relation to size of seeds

According to Ramage [50] the rates of transmission in barley trisomics were higher in smaller seeds than in the larger ones. Einset [17] examined the transmission of the extra chromosomes in maize trisomics and found a correlation between the chromosome length and the transmission rates; the trisomics for larger chromosomes threw a higher frequency of trisomics in their progenies than did the ones for shorter chromosomes. In *Datura* the chromosome length, however, had been shown to have no relation to the transmission rates; the smallest primary chromosome is transmitted to 32.7 percent of its offspring, while the third smallest showed only a 3 percent transmission [10]. According to Frost [19] working with *Matthiola* trisomics it is probable that the higher frequency of transmission of the extra chromosome is associated with the higher percentage of seed germination.

TABLE 8  
Size differences in two seed groups giving rise to diploid and trisomics

Types of trisomics	Length (mm)		Width (mm)		Thickness (mm)		Ratio L/W		Ratio L/T		Ratio W/T	
	2x	2x+1	2x	2x+1	2x	2x+1	2x	2x+1	2x	2x+1	2x	2x+1
Bush	12.0	12.1	2.51	2.18	1.85	1.61	47.8	55.5	64.9	75.2	1.36	1.35
Slender	10.4	10.4	2.32	2.03	1.61	1.23	44.8	64.6	64.6	74.6	1.44	1.65
Pale	10.0	9.8	2.57	2.50	1.66	1.40	38.9	39.2	60.2	70.0	1.55	1.79
Robust	11.3	11.1	2.52	2.32	1.48	1.20	44.8	47.8	76.4	92.5	1.70	1.93
Pseudo-normal	9.6	10.1	2.65	2.19	1.84	1.26	36.2	45.7	52.2	79.4	1.44	1.74
Purple	11.8	11.2	2.93	2.73	2.10	1.77	40.3	41.0	56.0	63.3	1.39	1.54
Semi-erect	12.7	12.9	3.01	2.63	2.24	1.84	42.2	49.0	56.7	71.0	1.34	1.43
Average	11.1	11.1	2.64	2.37	1.82	1.47	42.1	48.9	61.6	75.2	1.46	1.63
Diploid	11.9	—	2.77	—	1.94	—	42.9	—	61.3	—	1.42	—

In barley trisomics herein described, however, chromosome length and germination percentage seem not to be correlated with the rates of transmission (Tables 4, 9).

It is interesting to note that seed size has a close relation with the occurrence of trisomic plants from trisomic parentage. The length, width, and the thickness of seeds produced by selfing the parental trisomics were measured and sown individually and the germinated plants were classified as diploids or trisomics by chromosome counts or external morphology. Based on the results given above

the seeds were separated into two groups, namely, diploids and trisomics and the mean value of the two seed groups are calculated (Table 8). From the data presented in Table 9 it is apparent that there is no difference in the length of the two seed groups. However, significant difference was observed between the two seed groups in the width, thickness and the ratios of length/width, length/thickness and also width/thickness; the last is not so conspicuous (Table 8). The two seed groups are shown in Fig. 5 (cf. Tables 8, 9).

TABLE 9  
The number and percentage of trisomics in two seed groups of Bush  
and Slender differing in size

Types of trisomics	Seed size	Number of seeds			Trisomics obtained	
		Sown	Germinated	Percent	Number	%
Bush	Large	261	247	(94.63)	44	17.81
	Small	108	88	(81.48)	71	80.68
Slender	Large	128	108	(84.37)	17	15.74
	Small	46	20	(43.47)	18	90.00

Thus, the seeds from parental trisomics are easily separated into two groups by their measurements especially as to width and thickness. Further, the two seed groups are readily identified macroscopically; trisomic plants emerged from small, narrow seeds with smooth outline on the ventral side of the seed and the diploids from large and plump seeds with rough or uneven outline on the ventral side.

By this finding it is made easy to obtain trisomics from parental trisomics. Two instances in Bush and Slender are presented in Table 10. It is apparent in this table that most of the smaller seeds from both trisomic plants tended to give rise to trisomics, whereas the larger seeds produced only 15~17 percent of trisomics.

### 3. *Correlation between the occurrence of trisomics from autotriploids and the respective trisomic parents*

The frequency of occurrence of each trisomic type in the progenies of autotriploid not always corresponds with the frequency of transmission in progeny of the same trisomic type (cf. Tables. 2, 7). The trisomic type Pale appears most frequently in the progeny of autotriploid, but the trisomic was not so frequently recovered in the selfed progeny of Pale plants (Table 7). On the contrary, Robust and Pseudo-normal appeared very frequently in the selfed progenies of the same trisomic types, though both of which occurred only about 7 percent of all trisomics from the autotriploids (Table 7). A similar result has been found in tomato trisomics by Rick and Barton [56].

### 3. *Occurrence of exceptional types*

The occurrence of unrelated types are recorded in the offspring of trisomic plants in tomato [38], *Datura* [4, 5, 7, 8], *Nicotiana sylvestris* [21] and others. The primary trisomics of *Datura* have thrown exceptional types with the average



of 0.86 percent, ranging from 0.05 to 2.27 percent [10].

In barley, however, no unrelated types have been obtained in any of the seven primary simple trisomic types herein described with the exception of Purple from which an autotriploid (Fig. 14 h) was obtained.

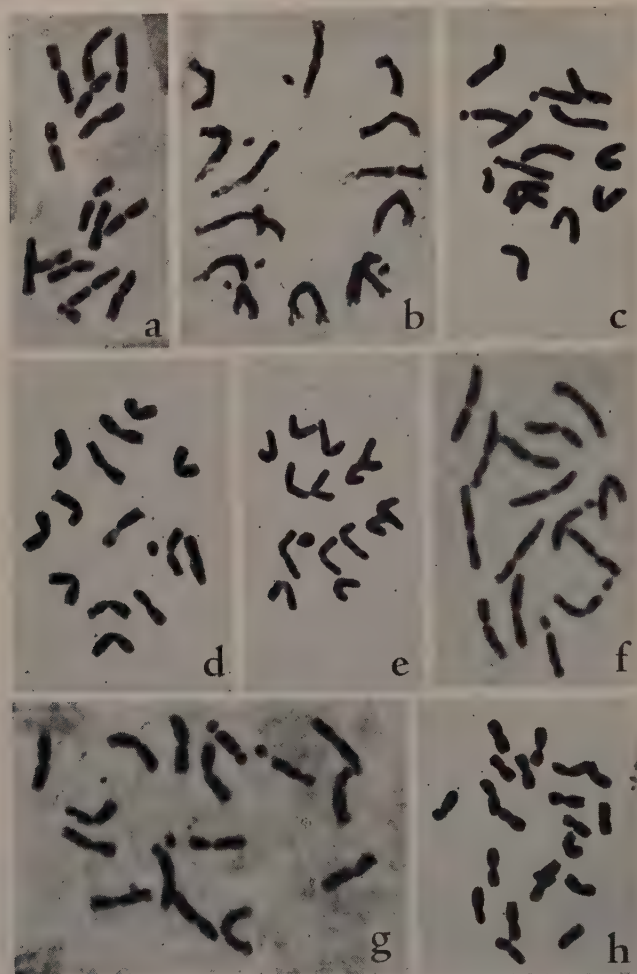


Fig. 14. Somatic chromosomes of aberrants in the progenies of primary simple trisomics.  $\times 1,000$ . a~e,  $2n=14+1$  fragment. a, a plant from Slender; fragment showing submedian constriction. b~h, plants from some Purple trisomics. b, 33-11; fragment having a large satellite showing that the fragment originated from chromosome 6, the extra chromosome of Purple. c, 608-10; showing a fragment with subterminal constriction. d, 610-1; showing a small fragment having subterminal constriction. e, 611-10; showing a ring fragment. f and g, Purple trisomic for changed chromosome 6 (f) and its tetrasomic (g): the changed chromosome 6 has a subterminal constriction and a large satellite indicating the origin of it from chromosome 6, the extra chromosome of Purple. h, 601-14; autotriploid from Purple showing  $2n=21$  chromosomes.

In contrast, other related chromosomal variants have been obtained frequently. In Purple, Bush, Slender, Pseudo-normal and probably all other types some plants with fragments besides  $2n=14$  or 15 chromosomes are observed. Fragments are varied in their size and shape (Fig. 14 a~e); ring fragments are observed in root tips of Pseudo-normal and Purple (Fig. 14 e). Simple trisomic plants having a deviating karyotype with far going structural changes of the extra chromosome are observed in the offspring of a Purple trisomic plant (Fig. 14 f). A tetrasomic plant with two altered chromosomes just mentioned was also obtained (Fig. 14 g) from a Purple trisomic as a sibling of the simple trisomics with changed extra chromosome.

Secondary trisomics were obtained in  $F_2$  populations of a Slender  $\times$  Colsess V cross; a ring trivalent was observed at meiotic diakinesis and MI of them.

Some other off type plants were occasionally observed which were not cytologically investigated.

#### VII. GENETIC STUDIES IN TRISOMIC HYBRIDS, WITH SPECIAL REFERENCE TO THE RELATION BETWEEN THE EXTRA-CHROMOSOMES AND THE GENETIC LINKAGE GROUPS

The genetic studies were carried out by testing  $F_2$  and  $F_3$  generations from trisomic  $F_1$  and  $F_2$  hybrids between trisomics and 4 linkage testers. The original material, *Hordeum spontaneum* C. Koch var. *transcaspicum* Vav., from which the trisomic types were derived is homozygous for 9 dominant and one recessive normal (+) characters allelic to 10 markers in the 4 linkage testers in the present experiments. The genes for the investigated characters are represented in Table 10.

TABLE 10

Marker genes, character pairs and their respective linkage groups involved in trisomics of *H. spontaneum* var. *transcaspicum* and 4 linkage testers

Linkage group	Character pairs	Analyzed genes in				
		Trisomic	Linkage testers			
		<i>H.s.t.</i> <sup>1)</sup>	Brachytic	<i>A<sub>6</sub>a<sub>6</sub>uzuz</i>	Colsess V	Minn 90-5
I	Non-six-rowed <i>vs.</i> six-rowed	<i>V</i>	<i>v</i>	<i>v</i>	<i>v</i>	— <sup>2)</sup>
II	Black <i>vs.</i> white lemma	<i>B</i>	<i>b</i>	<i>b</i>	<i>b</i>	—
III	Covered <i>vs.</i> naked caryopsis	<i>N</i>	<i>n</i>	<i>n</i>	—	—
IV	Hooded <i>vs.</i> awned	<i>k</i>	—	—	<i>K</i>	—
"	Blue <i>vs.</i> non-blue aleurone	<i>Bl</i>	<i>bl</i>	—	—	<i>bl</i>
V	Long <i>vs.</i> short haired rachilla	<i>S</i>	—	<i>s</i>	<i>s</i>	—
"	Rough <i>vs.</i> smooth awn	<i>R</i>	—	—	—	<i>r</i>
VI	Normal <i>vs.</i> "uzu"	<i>Uz</i>	—	<i>uz</i>	—	—
VII	Normal <i>vs.</i> brachytic	<i>Br</i>	<i>br</i>	—	—	—
"	Normal green <i>vs.</i> chlorina seedling	<i>F<sub>o</sub></i>	—	—	<i>f<sub>o</sub></i>	—

1) *Hordeum spontaneum* var. *transcaspicum*.

2) These characters (—) are the same as those of the trisomics or not tested as yet.

It is well known that the extra chromosomes present in the trisomic plants modify the genetic ratios for genes located on the chromosomes involved as has been shown in several plant species [9, 11, 16, 38, 39, 40, 43, 44, 50, 56]. Blakeslee and Farnham [11], in their study of trisomic inheritance in *Datura*, compared the segregation of character pairs in trisomics with the ratios expected on the basis of chromosome segregation. Further, theoretical considerations concerning trisomic segregation were made by Rhoades [54] and Lesley [40] who investigated the occurrence of chromatid crossing over and random chromatid assortment. On the basis of recent investigations of the primary trisomic types in tomato, Rick and Barton [56] calculated the frequencies expected in trisomic inheritance by assuming random complete chromatid association. Some of the expected ratios involved in this experiments are presented in Table 11.

TABLE 11  
Expected ratios in  $F_2$  (or  $F_3$ ) populations from trisomic and diploid hybrids

Genotype of $F_1$ hybrids			Trisomics		Diploids
			$AAa$	$Aaa$	$Aa$
Ratio of segregations in $F_2$ (or $F_3$ ) generation	Types of crossover	Portion of population	$A : a$	$A : a$	$A : a$
	Non-crossover	$2x$ $2x+1$	$8 : 1$ $18 : 0$	$5 : 4$ $7 : 2$	$3 : 1$
	Random, complete chromatid assortment	$2x$ $2x+1$	$8 : 1$ $44 : 1$	$5 : 4$ $11 : 4$	

If a gene is located on a chromosome present in disomic dose,  $F_2$  segregation will be in a Mendelian  $3A:1a$  ratio, where  $A$  is a complete dominant over  $a$  (Table 11).

If random complete chromatid association takes place after chromatid crossing over, the ratio of gametes of  $F_1$  hybrids having the genotype of  $AAa$  will be, according to Lesley [40]:

$$AA : Aa : aa : A : a = 6 : 8 : 1 : 10 : 5$$

From selfed  $F_1$  trisomics with the genotype  $AAa$  the following ratios of segregations are expected; namely,  $8A:1a$  for the diploid portion and  $44A:1a$  for the trisomics (Table 11). Though a preliminary experiment showed that the extra chromosome is not transmitted through the pollen (cf. Table 7), the segregating ratios in  $F_2$  generation should still be unchanged. The expected ratios from the  $F_1$   $Aaa$  trisomics were also calculated by the assumption of chromatid segregation (Table 11). The segregations of the character pairs in  $F_2$  (or  $F_3$ ) generation were compared with the expected ratios given in Table 11 (cf. [83]).

The trisomic plants were always used as female parents in all crosses with the 4 linkage testers shown in Table 10, because the extra chromosomes are usually transmitted only through the egg (Table 7). Some other procedures in treating the results of genetic studies has been given in previous paper [83].



Most of the results of the genetic studies in trisomic barleys have already been reported in previous paper [83, 86]. So, only the summary of the results of the genetic studies will be presented in this paper (Tables 12, 13)

Nine character pairs tested against the diploids (Control) segregated in a simple Mendelian ratio, 3A:1a [83].

Up to the present time tests have been made of the segregation of 10 allele pairs for seven primary simple trisomic types in 66 populations among the possible 70 cross combinations. Among them, 46 populations proved to fit very well with disomic expectation with the exception of *Kk* allele pair in linkage group IV in 6 trisomic types [83, 86]. The following 5 out of 66 populations were not analyzed sufficiently because the number of plants in those populations was too small to be tested for segregating ratio: *Kk*, *Ss* and *F<sub>6</sub>f<sub>6</sub>* in Robust and *Nn* and *Brbr* in Pseudo-normal. Four populations, namely, *Bb* and *Ss* in Bush and *Nn* and *Brbr* in Semi-erect, were slightly deviated from disomic expectation at 5% level, however, the possibility of trisomic inheritance of the 4 tested genes was ruled out because an appreciable number of trisomics were recessive homozygotes and the  $\chi^2$  values were fitted very well with disomic expectation at 2% level.

The remaining 11 populations which were significantly deviated from disomic expectation even at 1% level were tested for trisomic expectation after dividing the populations into diploid and trisomic portion. As a result the possibility of trisomic segregation in 2 populations, *Bibl* in Bush and *Nn* in Pale, was also ruled out, because an appreciable number of trisomics were recessive homozygotes and  $\chi^2$  values significantly deviated from trisomic expectations in both diploid and trisomic portions (Table 12; [83, 86]).

Nine populations which significantly deviated from disomic expectations segregated no homozygous recessive trisomics with one exception of Semi-erect segregating for *Ss*, in which two homozygous recessive trisomics were observed among 47 trisomics [83]. In all 9 genes just mentioned a good fit to the expected trisomic ratios was observed in the diploid and/or trisomic portions. As a result nine allele pairs belonging to the seven genetic linkage groups can be associated with the respective trisomic except for Purple (Tables 12, 13). Bush, thus, appears to carry on its extra chromosome two previously established genetic linkage groups, III and VII, as has been reported by Kramer *et al.* [37].

An *F<sub>3</sub>* test for *Kk* in linkage group IV was carried out with Robust as shown in previous paper [83, 86]. Five *F<sub>3</sub>* families were obtained from selfed *F<sub>2</sub>* trisomic plants which had so-called subjacent hood instead of normal hood, owing to the presence of an extra Robust chromosome (Tsuchiya unpublished data). In none of the populations the disomic ratio was observed and the  $\chi^2$  values fitted very well with the trisomic expectation in both diploid and trisomic portion [86]. This finding gives a decisive evidence of trisomic inheritance of *Kk* allele pair in the Robust trisomic, which agrees well with the fact that the allele pair of *Bibl* on the same linkage group IV shows also the trisomic inheritance in Robust (Tables 12, 13; [83, 86]).

The results obtained in the present experiments are presented in Table 12



TABLE 12

Summary of tests against disomic and/or trisomic ratio of ten marker genes involved in respective linkage groups of barley

Types of trisomics	Marker genes in respective linkage groups tested against										
	Trisomic ratio	Disomic ratio									
		I	II	III	IV	V	VI	VII			
Bush	$n$ (III)	$v$	$B$	$*$	—	$bl$	$s$	$r$	$uz$	$*$	$*$
"	$br$ (VII)										
"	$f_c$ (VII)										
Slender	$v$ (I)	$*$	$B$	$n$	$K$	$bl$	$s$	$r$	$uz$	—	$f_c$
Pale	$uz$ (VI)	$v$	$B$	$n$	$K$	$bl$	$s$	$r$	$*$	$br$	$f_c$
Robust	$K$ (IV)	$v$	$B$	$n$	$*1)$	$*$	—	$r$	$uz$	$br$	$f_c$
"	$bl$ (IV)										
Pseudo-normal	$B$ (II)	$v$	$*$	—	$K?$	—	$s$	—	—	—	$f_c$
Purple	— —	$v$	$B$	$n$	$K$	$bl$	$s$	$r$	$uz$	$br$	$f_c$
Semi-erect	$s$ (V)	$v$	$B$	$n$	—	—	$*$	$*$	$uz$	$br$	$f_c$
"	$r$ (V)										
Diploid (Control)	— —	$v$	$B$	$n$	$K$	—	$s$	$r$	$uz$	$br$	$f_c$

1)  $F_3$  data, all others  $F_2$  data. \* Trisomic ratio. — Test not yet completed.

and the relationships between the trisomics and the genetic linkage groups are shown in Table 13. From the data obtained in the present experiments the following conclusions may be drawn:

1. The extra chromosome of the 4 trisomic types, Slender, Pseudo-normal, Robust, and Pale carry the genetic linkage groups I ( $Vv$ ), II ( $Bb$ ), IV ( $Blbl$ ,  $Kk$ ), and VI ( $Uzuz$ ), respectively.

2. Bush carries on its extra chromosome the two genetic linkage groups previously established, III ( $Nn$ ) and VII ( $Brbr$ ,  $F_c f_c$ ); the result is in accord with that furnished by Kramer *et al.* [37].

3. The extra chromosome of Semi-erect (chromosome 7 or Burnham's  $d$  with smaller satellite) carries the genetic linkage group V ( $Ss$ ). This was already assumed by Kramer *et al.* [37] and Burnham [13] but questioned by Burnham and Hagberg [14] and Hagberg [25] (cf. [80]).

4. The extra chromosome of Purple (chromosome 6 with the larger satellite) does not carry any one of the known genetic linkage groups, I~VII, which have been previously established by the ordinary genetical methods (cf. [51]).

5. As a result 9 of 10 allele pairs tested each of them belonging to one of the seven genetic linkage groups could be associated with the respective trisomic type with the exception of Purple in which the gene for the examined characters could not be associated with the extra chromosome (Tables 12, 13).

6. All the results described herein have led to the conclusion that the number of linkage groups in barley established by the ordinary genetic methods as 7 is only 6. An other linkage group may be associated with the chromosome 6

TABLE 13  
Interrelationships between trisomics, linkage groups, and chromosomes of barley

Types of trisomics	Analyzed genes	Linkage groups	Chromosomes	
			by Burnham	Standard*
Bush	<i>n, br, f<sub>e</sub></i>	III (+VII)	<i>b</i>	(1)**
Slender	<i>v</i>	I	<i>f</i>	(2)
Pale	<i>uz</i>	VI	<i>c</i>	(3)
Robust	<i>bl, K</i>	IV	<i>e</i>	(4)
Pseudo-normal	<i>B</i>	II	<i>a</i>	5
Purple	( <i>ec, x<sub>n</sub>, o</i> )****	VII****	<i>g</i>	6
Semi-erect	<i>s, r</i>	V	<i>d</i>	7

\* Hagberg and Tjio (1951).

\*\* Numerals in parentheses ( ) show that the extra chromosome of the respective trisomic type is not yet determined by karyotype analysis. The remaining 3 chromosomes without parentheses are determined by karyotype analysis of respective trisomic types.

\*\*\* Identification of the three chromosomes are not yet completed.

\*\*\*\* Not yet established by trisomic tests but translocation tests.

with large satellite as suggested by Kramer *et al.* [37] and Burnham [13]. In fact recently several genes have been located on the chromosome 6 by the study of reciprocal translocations ([51], Kramer, and Burnham, unpublished data).

### VIII. DISCUSSION AND CONCLUSION

The establishment of all the possible types of primary simple trisomic plants corresponding to the haploid number of chromosomes is desirable in trisomic studies, but success was achieved only in a few plants. *Datura* is the first instance in which all the 12 possible types have been established [4, 5, 6, 7, 8, 10]. In tomato [38, 39, 40, 56], maize [17, 18, 43, 44, 55], *Matthiola* [20, 41], *Oenothera* [16, 33, 52, 53], *Nicotiana* [21, 22, 61], spinach [87, 88], *Antirrhinum* [89], and others, all or almost all of the expected primary simple trisomics have been obtained with or without sufficient cytogenetic evidences for the independence of the different types.

As the possible sources of the simple trisomic plants, Blakeslee [4, 5, 6, 7, 8], Blakeslee and Avery [10], Goodspeed and Avery [21] have suggested the following 5:

- 1) Normal diploids (spontaneous occurrence)
- 2) Trisomic plants (unrelated types from parental trisomics)
- 3) Asynaptic plants (uneven distribution of chromosomes at meiosis)
- 4) Irradiated progenies (from interchange heterozygotes and other chromosomal variants)
- 5) Triploid progenies

In barley the primary simple trisomics have been obtained from all the sources described above except for 2) [82]. It is well known in many plants that

triploids are one of the best sources of trisomics. In fact, they have been obtained most frequently from triploids in *Datura*, *Zea*, *Lycopersicon*, *Nicotiana*, *Avena*, spinach, *Antirrhinum* and many others. In barley, however, the triploids have not been used as the source of trisomics because of the difficulty in inducing triploids [28, 63, 70, 72, 73, 75, 79] with some exceptions [47, 71, 72, 77]. In Table 8 of the previous paper [82] the frequency of trisomics from various sources in barley are presented.

Trisomics have been obtained in barley from autotriploids by the present author [71, 72, 77, 78, 80, 82, 83] and from triploid hybrids by Kerber [36]. The frequency of trisomic plants is extremely high in the progeny of triploids compared with the other sources such as translocation heterozygotes and asynaptic plants; about one half of triploid progenies were simple trisomics (Table 1) while the other sources produced only 1 to 3 percent [82]. Further the trisomic plants obtained from autotriploids were primary types in almost all of the plants (Tables 2, 5).

From the double and triple trisomic plants primary simple trisomics were also frequently obtained (Table 2). Since the double and triple trisomics which were obtained as siblings of simple trisomics in the progenies of autotriploids were relatively vigorous and more or less seed-fertile, they are considered to be one of good sources of simple trisomics in barley. The same was already reported in *Datura* [7, 8, 10], *Nicotiana* [21, 22], *Lycopersicon* [38, 39] and others. Thus the triploids have proved to be the most useful source of trisomics in barley [71, 72, 77, 82].

Trisomics in barley have been obtained several times [82]. However, a complete series of the seven primary simple trisomic types has not been reported (cf. [63]). A complete series of trisomic types which is dealt with in this paper has been for the first time established in 1954 from progenies of an autotriploid wild barley, *Hordeum spontaneum* var. *transcaspicum* [77]. Next year, Ramage [50] reported the establishment of all seven primary simple trisomic types from the translocation heterozygotes of a six-rowed cultivated variety, Mars.

As mentioned above a complete series of primary simple trisomic types was established by Ramage [50] and by the present author [77, 78, 82, 83] from different sources. The materials used by the two authors are partly similar as to their morphology and other characteristics, but are strikingly different in seed fertility. The main characteristics including the seed fertility of these two trisomic stocks are represented in the previous paper [82]. The differences between these two stocks may be attributed mainly to the differences of (1) the original materials from which the trisomics were secured and (2) the process of trisomic production, namely, from translocation heterozygotes by Ramage [50] and autotriploids by Tsuchiya [82].

The materials used by Ramage [50] was a cultivated six-rowed variety, Mars, *Hordeum vulgare*, which may have various mutant characters which raised in the course of cultivation and breeding during long period. While the materials used by the present author was a wild species, *H. spontaneum*, which has many wild characters constant in their expression. It has been bred true in successive years thereby may be said to be highly stable. The vigorous growth and good



expression of the distinctive and diagnostic characteristics by which the trisomics are readily distinguished from each other and also from normal diploids of Tsuchiya's stocks may be attributed to this original material taken from a state of nature.

The high sterility found in Ramage's trisomics may be mainly ascribed to cytological abnormalities having been originated from X-ray induced segmental interchanges which may have brought about disadvantageous changes in chromosomes and/or genes, whether visible or cryptic. The high sterility and weak expression of distinctive characteristics in Ramage's trisomic stocks may be partly attributed to having their origin in a cultivated variety. Similar results have been reported by the present author for some trisomic plants secured from a two-rowed cultivated variety, Early Golden Melon, which showed sometimes complete sterility in selfing though they showed a relatively high fertility when crossed with normal diploids [72].

Thanks to the high seed fertility, high transmission rates and more or less vigorous growth in all trisomic types herein described (see Figs. 1 and 3) they have fairly readily been maintained in homozygous condition, though there appeared occasionally some cytological aberrants such as secondary trisomics, autotriploids or plants with fragments and other structural changes (Fig. 14) in some trisomic types.

The trisomic plants herein described are classified into seven different types based mainly on their morphological as well as physiological characteristics. The extra chromosomes by which the trisomic types are distinguished should be accurately defined as has been done for *Zea*, *Lycopersicon* and some others.

Although the barley chromosomes are very similar to each other as to size and shape, especially in the 3 or may be 4 longest chromosomes but two pairs of satellited chromosomes are easily distinguished. As Fig. 6 shows, two trisomic types, Purple and Semi-erect, can be clearly cytologically identified by their karyotypes thanks to the appearance of a large or small satellite in their extra chromosomes: Purple is trisomic for chromosome 6 which has a large satellite (Fig. 6g) and Semi-erect for chromosome 7 with the small satellite (Fig. 6h). These results are also supported by the study of nucleolus-chromosome relationships at diplotene and diakinesis of meiosis (Figs. 12, 13; Table 6) and are in agreement with the results of genetic studies [78, 83, 86] (Tables 11, 12). Pseudonormal was shown to have chromosome 5 (the shortest one or Burnham's *a*) in triplicate condition (Fig. 6f). The other 4 trisomics are not yet accurately determined as to the karyotypes of their extra chromosomes. However, the independence of these trisomic types is to some extent cytologically indicated (see Figs. 7~10 and Tables 5, 6). The chromosome configurations and the types of the trivalents varied with different trisomic types at both diakinesis and metaphase I (Table 5). The differences of nucleolus-chromosome relationship as well as nucleolus conditions may also contribute to cytological identification of the seven trisomic types (Table 6 and Figs. 7~13).

The study of chromosome behavior at diplotene and diakinesis of meiosis showed that the trisomic plants are very useful for the study of nucleolus-chromosome relationships as has been previously reported by the present author



for barley [65, 74, 80, 84] and by Lin [42] for maize. The present investigation revealed a nucleolus organizing capacity of chromosome 7 with the smaller satellite (Fig. 13, Table 6). This was suggested by the present author working with trisomics and translocation heterozygotes of barley [74, 80] and clearly shown by Sarvella *et al.* [60] in the study of pachytene chromosomes of barley. The nucleolus organizing capacity of chromosome 6 or so-called nucleolus chromosome (Burnham's *g*) with the large satellite was shown to be very strong, especially when the chromosome is involved in the triplicate state as in Purple (Table 6 and Fig. 12). The existence of third and fourth nucleolus-organizing chromosomes is recognized in some sporocytes of Purple, since two (Fig. 13 c) or three bivalents as well as the trivalents (Fig. 13 f) touch the 3 or 4 nucleoli. This finding also showed that the presence of an extra chromosome 6 may favor association of the nucleolus with the nucleolus organizing chromosomes even if the nucleolus organizing capacity is very weak ([84], Tsuchiya unpublished data).

Some certain cytological abnormality characteristic of a trisomic type may afford an evidence for the independence of the type concerned; for instance, partial asynapsis in Slender (Fig. 8 d, g; cf. [85]) and the types of trivalents in Bush and Semi-erect (Table 5) may help to distinguish three types from the others as well as from each other. Multiploid sporocytes were observed only in Pale (Fig. 9 g~j) though the Pale plants not always showed the syncyte formation [85].

The study of meiotic pairing of chromosomes in trisomic  $F_1$  hybrids of trisomic stocks with Burnham's translocation testers having the identified chromosomes [12, 13, 14, 15] may, further, furnish cytological evidences for the independence of each of the seven trisomic types (Tsuchiya unpublished).

As described above, it may be said that cytological evidences for the independence of the seven trisomic types have become to a considerable extent available, though they are in some cases not conclusive. Also genetic identification provides a powerful evidence for the independence of the trisomic types here described. As has been shown above (Tables 12, 13) the results of genetic studies are very clear-cut and strongly support the independence of the seven types (Table 13; [83, 86]).

The identification of the individual barley chromosomes with the genetic linkage groups is certainly of scientific interest and one of the most important objects of this study. Some attempts to determine these relationships have been made with considerable success on the basis of reciprocal translocations [12, 13, 15, 29, 30, 31, 34, 37, 50 51].

The trisomic method is, as was confirmed by the present author's experiments, very useful for testing the independence of genetic linkage groups and associating the individual chromosomes with the genetic linkage group, provided that the types of the extra chromosomes are accurately determined (cf. [18, 56]).

The interrelationships between trisomics, linkage groups and chromosomes in barley are represented in Table 13. It shows that chromosome 6 carries no genetic linkage group previously established. This is interesting considering the fact that the two genetic linkage groups, III and VII, are located on one of the 3 longest chromosomes, Burnham's *b* which is the extra chromosome of Bush

(Tables 12, 13). Thus the known genetic linkage groups have been reduced from 7 to 6 in barley [78, 80, 82, 83, 86]. Recently, however, Ramage and Suneson [51], Kramer (unpublished), Burnham (unpublished) and some other workers located several new genes on the chromosome 6 by a study with reciprocal translocations; *ec* (early) by Ramage and Suneson [51], *x<sub>n</sub>* (xantha seedling) by Kramer, *o* (orange lemma) by Burnham. As a result seven genetic linkage groups corresponding to the haploid chromosome number of barley are again reinstated and the independence of the seven linkage groups is definitely established (Table 13).

## IX. SUMMARY

All seven primary simple trisomic types corresponding to the haploid number of chromosomes in barley were established from the progeny of autotriploids of a wild two-rowed barley, *Hordeum spontaneum* C. Koch var. *transcaspicum* Vav. The results of observational and experimental studies are summarized as follows:

1. A total of 59 simple trisomics were originally obtained in the progeny of autotriploid plants of *Hordeum spontaneum* var. *transcaspicum*. A number of similar trisomics were also obtained in the progeny of double and triple trisomic plants which were siblings of the simple trisomics.

2. The simple trisomics appeared most frequently among various chromosomal types in the progeny of autotriploids; 47 percent were simple trisomics, 23 percent were diploids, 18 percent were double trisomics, 5 percent were 17-chromosome plants; moreover 18-, 20-, and 25-chromosome plants occurred, 1 percent each. Thus the autotriploids may be said to be one of the best sources of primary simple trisomics in barley.

3. According to their morphological characteristics the simple trisomics were classified into the following seven types: Bush, Slender, Pale, Robust, Pseudo-normal, Purple, and Semi-erect.

4. The seven types are readily distinguished from the normal diploids and also from each other by their distinctive traits which are expressed at various growth stages from a very early seedling stage until maturity in almost all of the plant organs. Identification of the trisomics and their diploid sibs can also be made from the size of the seeds.

5. The trisomic plants here obtained showed surprisingly high pollen fertility (93~97%) with the exception of Pale (72%).

6. Seed fertility was also relatively high in all the seven types though it varied from year to year, from type to type and also with methods of breeding.

7. Germination capacity of seeds was very high in all the seven types when selfed, and of those obtained from  $(2x+1) \times 2x$  and  $2x \times (2x+1)$ ; the average for the seven types in 3 cases was 87.3 percent ranging from 80.3 percent for Pale and Slender to 91.9 percent for Bush.

8. By karyotype analysis the extra chromosomes were determined in 3 of the 7 types: Pseudo-normal is trisomic for chromosome 5 (the shortest of the complement or Burnham's *a*), Purple is trisomic for chromosome 6 (Burnham's

*g*) with larger satellite and Semi-erect is trisomic for chromosome 7 (Burnham's *d*) with the smaller satellite. In the other 4 trisomics, Bush, Slender, Pale and Robust, the extra chromosomes have not yet been identified accurately; each has one of the longest 4 chromosomes without satellite.

9. The chromosome behavior at meiosis of the seven trisomic types is similar to each other and also to that in other trisomic plants. Trivalents or univalents appeared at meiosis followed by some disturbances of the division process and occurrence of lagging chromosomes at AI, TI, AII and TII and micronuclei at interkinesis and tetrad stage. Pollen abortion and reduced seed fertility may be resulted from such cytological abnormalities.

10. Nucleolus-chromosome relationships have been investigated in detail in all seven trisomic types with the following results:

a. The nucleolus organizing capacity of chromosome 6 is the strongest which is followed by chromosome 7, since Purple trisomic for chromosome 6 showed the highest number of nucleoli (4) per sporocyte and highest frequency of sporocytes with 2~4 nucleoli (12%).

b. In Semi-erect 2 or 3 nucleoli occurred in about 6 percent of the sporocytes analyzed. The trivalent chromosomes in Semi-erect touch the nucleolus which strongly indicates a nucleolus organizing capacity of chromosome 7 with the small satellite.

c. In some other trisomic types, sporocytes with 2 nucleoli touched by 2 or 3 different chromosomes including a trivalent have been sometimes observed.

d. In almost all of the trisomic types a lemon-shaped nucleolus was observed to be touched by two different kinds of bi- or trivalent chromosomes from the opposite sides, indicating their nucleolus organizing capacity.

11. The frequency of various chromosome configurations and the types of trivalents differ with different stages of meiosis: at diakinesis  $1_{III}+6_{II}$  and frying-pan trivalents predominate, but they become less frequent at MI as number of  $7_{II}+1_I$  and chain trivalents increase.

12. Chromosome configurations and other behaviors are also different according to the trisomic type; this may be helpful in testing the independence of the primary simple trisomic types. Partial asynapsis in the trisomic Slender may be a direct effect of the extra chromosome. As to the syncyte formation in Pale it is not sure whether it is a specific phenomenon for this type.

13. The effects of extra chromosomes are expressed in many morphological characters and cytological behaviors. Some are strongly but some others are weakly expressed; some are qualitative and others quantitative; some are expressed fairly well at very early seedling stage and others at maturity or throughout the whole period of growth.

14. Rates of transmission of the extra chromosome are fairly high through the ovules in all the seven types but they vary with the types. No transmission was observed through the pollen. Trisomic plants occurred more frequently from small seeds than from the large ones.

15. High seed fertility, high germination capacity and high transmission



rates as well as vigorous growth and fairly well expressed effects of the extra chromosomes made a successful performance of the genetic experiments possible.

16. The results of the genetic studies of trisomic plants strongly support the specificity of each trisomic type and are in accord with the results of morphological and cytological studies.

17. By the genetic studies of these trisomic types the independence of 6 genetic linkage groups has been established and a definite evidence was obtained for locating the two genetic linkage groups, III (*Nn*) and VII (*Brbr*, *Fefe*), on one and the same chromosome, the extra chromosome of Bush.

18. The trisomics, Slender, Pale, Robust and Pseudo-normal are shown to carry the genetic linkage groups, I (*Vv*), VI (*Uzuz*), IV (*Bibl*, *Kk*), and II (*Bb*), respectively, on their extra chromosomes.

19. Semi-erect definitely showed to carry the genetic linkage group V (*Ss*) on its extra chromosome, the chromosome 7 with small satellite (Burnham's *d*).

20. The extra chromosome of Purple (chromosome 6 or Burnham's *g*) with large satellite is shown to carry probably a new linkage group to which several genes have been recently located.

21. The usefulness of trisomics for the independence test of genetic linkage groups and also for the establishment of the expected seven groups in barley is considered from cytogenetic points of view.

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## THE ROLE OF PROTEIN AND RIBONUCLEIC ACID IN THE DIFFERENTIATION OF FERN GAMETOPHYTE

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In the majority of *Filicales* ferns, especially in those belonging to *Polypodiaceae*, unicellular spores differentiate first into one rhizoidal and one protonema cell at germination. Then the protonema cell enters in a phase of successive cell divisions which occur in one direction, resulting in so called filamentous protonema. This growth pattern will be referred to as "the primary one-dimensional growth". After a definite time lapse, the direction of cell division of the apical cell of the protonema is converted from longitudinal to transverse. The subsequent division of these cells takes place in all directions but in the same plane, forming a plate-like tissue of monocellular layer. This growth pattern will be called "two-dimensional". The plate-like organism in the two-dimensional growth, i.e. young prothallium, develops first to a heart-shaped prothallium, and finally to a mature prothallium provided with antheridia and archegonia.

In *Dryopteris erythrosora* which has been studied in the present paper, spores begin to germinate five days after dusting in the culture medium under standard conditions. The primary one-dimensional growth of the protonema proceeds up to a seven-cell stage, which is followed by the two-dimensional growth beginning approximately 25 days after germination. About 100 days are required until the mature prothallium is completed.

The experiments to be described in this paper concern the artificial control of differentiation of this organism by some chemicals which are known to interfere with the synthesis of protein and nucleic acid. The relation of ribonucleic acid (RNA) to the mode of growth has also been investigated.

It was shown that by the action of some amino acid analogs or 8-azaguanine, the growth pattern is converted from two-dimensional to one-dimensional. Further, it was demonstrated that the one-dimensional growth, whether it was normal or artificial, was characterized by a decrease of protein concentration, whereas the two-dimensional growth was accompanied by its rapid increase. Because of the apparent involvement of protein metabolism in the two-dimensional growth which is affected by 8-azaguanine, we come to the idea that RNA is in some way connected with this type of growth. Several aspects of the relation between RNA and the two-dimensional growth have then been studied. It was shown that the added 8-azaguanine is actually taken up by RNA in the cell, when the mode of growth is converted from two-dimensional to one-dimensional. Studies on the correlation of the quantitative as well as qualitative changes of RNA in the cell with the mode of growth strongly suggest that a special RNA fraction which is sensitive to the action of 8-azaguanine is closely related to the

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two-dimensional growth.

### MATERIAL AND METHOD

Spores of *Dryopteris erythrosora* were dusted in the culture in Petri dishes which were then placed under diffused day light and kept at 27°C.

As the standard culture medium, a modified Knop's solution was used: 2.32 g  $\text{KH}_2\text{PO}_4$ ; 2.994 g  $\text{Na}_2\text{HPO}_4$ ; 0.2 g  $\text{Ca}(\text{NO}_3)_2$ ; 0.024 g KCl; 0.05 g  $\text{MgSO}_4$  and  $\text{FeCl}_3$  trace, made up to 1000 ml with glass-distilled water. The pH of the solution was kept at 6.3. The culture medium was refreshed every week and the morphological changes of protonema or prothallium were checked every fifth day.

Changes in the absolute amount of dry weight or in the protein nitrogen content of a single spore during the course of development was estimated in the following way: Eleven cultivations of spores in separate Petri-dishes were made under our standard conditions, each of cultures containing 1 mg of spores. The contents of each dish were quantitatively collected successively 0, 10, 20, 25, 40, 50, 60, 70, 80, 90 and 100 days after the germination, and analysed for dry weight or protein nitrogen. It was found that 1 mg portion contains about 5500 spores, and dry weight or protein nitrogen content derived from single spore was calculated accordingly.

For the estimation of protein nitrogen, materials were collected from one to several Petri-dishes at a desired stage. All collected material was carefully washed several times with distilled water, blotted, weighed, and kept in 80 per cent ethanol for six hours. After centrifugation it was homogenized with a glass homogenizer and suspended in cold 10 per cent trichloroacetic acid (TCA) solution and left undisturbed for 15 hours at 2°C. The precipitate obtained was suspended in 5 per cent TCA and heated at 90°C for 20 min. to remove nucleic acids. The precipitate washed with 5 per cent TCA was employed as the protein fraction. For the nitrogen estimation, a modified Levy and Palmer's method (Yagi, 1951) was used.

For the  $^{14}\text{C}$ -8-azaguanine incorporation experiment, the young prothallium 10 days after the beginning of the two-dimensional growth transferred into the culture medium containing 0.1 mc 2- $^{14}\text{C}$ -8-azaguanine with specific activity of 500 mc/mol (final concentration, M/12,500) and was cultured for 30 days. A sample of about 100 g. in wet weight was obtained. RNA prepared by the method of Amano *et al.* [1] was further hydrolyzed with 0.1 N KOH at 80°C for 20 minutes to yield a mixture of mononucleotides which were further separated with Dowex-1 ion exchange chromatography. The technique previously described was followed, except for an additional elution by 110 ml. 6 N formic acid (Mandel *et al.*, 1957) to obtain 8-azaguanic acid, if present. Under these conditions contaminated  $^{14}\text{C}$ -8-azaguanine, if any, should be eluted earlier than cytidylic acid.

Content of each tube was plated on a stainless steel planchette and dried to form a very thin film. Radioactivity was counted at a thickness of negligible self-adsorption with a Geiger-Müller tube and a scaler.

For the estimation of RNA content, materials collected from several Petri-dishes at a desired stage were washed several times with distilled water, blotted,

weighed, and homogenized with the same volume of 0.2 M phosphate buffer (pH 7.1) in the cold. Cold 100 per cent TCA solution was then added to the homogenate at a final concentration of 10 per cent. After about 10 minutes, the homogenate was centrifuged at  $7,000\times g$  for 20 minutes, and the precipitate obtained was successively treated with a large volume of cold 5 per cent TCA (4 times), ethanol saturated with sodium acetate (5 times), 3:1 ethanol-ether mixture (once), ether (twice), and finally dried in the air. About 500 mg. dry powder were suspended in 10 ml. of 0.02 per cent ribonuclease solution dissolved in 0.05 M pH 7.2 Tris buffer. After the incubation at  $37^{\circ}\text{C}$  for 18 hours, the suspension was centrifuged at  $12,000\times g$ . for 20 minutes. The precipitate was washed with 5 ml. of 0.05 M Tris buffer at pH 7.2. The supernates were combined and heated with 0.1 N KOH at  $80^{\circ}\text{C}$  for 20 minutes. This solution was then acidified with 60 per cent perchloric acid (final concentration, 0.2 N) in the cold to precipitate protein and DNA. The supernate was neutralized with 6 N KOH (pH 7.5–8.0). The resulting precipitate of  $\text{KClO}_4$  was removed by centrifugation. The supernate contains mononucleotides of RNA. The mononucleotide mixture so obtained was further separated on the Dowex-1 column (Osawa *et al.*, 1958).

The procedure used for the analysis of nucleotide composition of RNA was essentially the same as that described under "estimation of RNA". The solution containing about 1.5 to 6 mg. RNA mononucleotides was applied on the column.

## RESULTS

### 1. Changes in protein contents

The concept that differentiation of the cell or tissue involves the synthesis of new kind of protein has now been generally accepted. It may therefore be of interest to know if the onset of the two-dimensional growth is characterized by a change in the protein metabolism.

In the first place, changes in the protein content during the formation of the young gametophyte have been followed. Representative data are shown in Fig. 1. From this it is clear that up to the end of the primary one-dimensional growth, only a slight increase in the absolute amount of protein is detectable. As soon as the two-dimensional growth begins, a sudden increase in the protein content becomes apparent which continues throughout the two-dimensional growth. As an accurate cell count in later stages of the two-dimensional

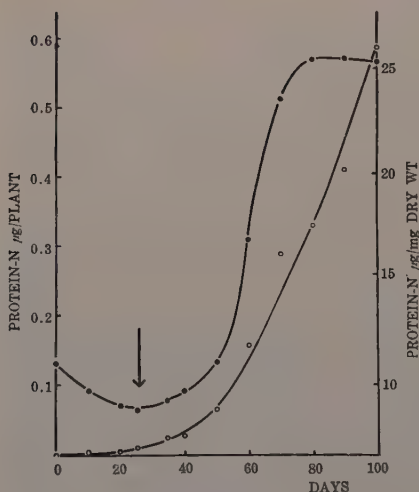


Fig. 1. Changes in protein content per dry weight (●—●) and per single plant (○—○) during the course of germination and prothallium formation in *Dryopteris erythrosora*. Arrow indicates the beginning of the two-dimensional growth.



growth is technically not possible at present, an estimation of the protein content per cell was not undertaken. However, if the protein content per dry weight is taken as an approximate index of the protein concentration in the cell, the one-dimensional growth is characterized by slightly decreasing protein concentration, whereas the two-dimensional growth is accompanied by a rapid increase in protein concentration.

As seen in the later section of this paper, such relationship between the protein concentration and the type of growth holds true when the mode of differentiation is artificially altered.

## 2. *Effects of amino acid analogs*

In the foregoing section, it was mentioned that the two-dimensional growth is characterized by a distinctly higher rate of protein synthesis. It may therefore be expected to have a preferential inhibition of the two-dimensional growth by affecting the synthesis of protein characteristic for it. For this purpose, the effect of amino acid analogs, ethionine and 5-methyltryptophane, was tested. In a preliminary experiment, each analog was introduced into the culture medium in a wide range of concentration (M/50 to M/5000). The developmental stages, in which analogs were added, involved spores, protonema of five-cell stage, and young prothallium 10 days after the two-dimensional differentiation. The morphological changes were followed under the microscope for about 50 days after the addition of analogs. Almost no effect on the morphological features was observed at a concentration below M/1000 for both analogs. Concentrations above M/100 cause death of organism shortly after the addition. At the concentration of about M/500 preferential inhibition of the two-dimensional growth was clearly demonstrated without any effect on the one-dimensional growth, and the two-dimensional growth was always converted into the one-dimensional growth.

When the analogs were added to the culture of ungerminated spores, germination and the subsequent primary one-dimensional growth were not affected. However at the seven-cell stage, when in the control the beginning of the two-dimensional growth is preceptible, no sign of the latter was noted in the experimental culture containing analogs. The apical cell kept proliferating in the one direction, forming a long filamentous protonema even when examined 50 days after the germination.

A comparable result was obtained when the analogs were added 10 days after the two-dimensional differentiation: the apex of the young plate-like prothallium at this stage is composed of three to six cells (only these cells proliferate under our experimental conditions), and by addition of analogs these apical cells changed in growth pattern from the two-dimensional to one-dimensional type.

As already mentioned, during the primary one-dimensional growth, no increase, but a slight decrease in the protein content per dry weight was noted. It would therefore be reasonable to assume that the "artificial" one-dimensional growth induced by amino acid analogs is characterized by the protein metabolism similar to that of the primary one-dimensional growth. To test this idea, the following experiments were performed. Ethionine or 5-methyltryptophane (M/500)



was added in the culture medium containing the prothallium 10 days after the two-dimensional growth; after 15 days all cultures were found to contain long filamentous protonema growing in the one-dimensional direction. Comparison of its protein concentration with those of the initial prothallium and with those of the young plate-like prothallium cultured for the same period without adding analogs (i.e. the control) made it clear that in the control a sharp increase in the protein content occurs, whereas in the culture containing analogs there is a slight decrease in the protein content (Table 1). A tentative conclusion from this observation is that the decrease of the protein content is one of the characteristics of the one-dimensional growth whether it is normal or artificially induced.

TABLE 1

Effects of ethionine and 5-methyltryptophane on the differentiation and the protein content of young prothallium of *Dryopteris erythrosora*

For explanation, see text

	Starting material	Control	Experimental	
			Ethionine	5-methyl-tryptophane
Protein-N: $\mu\text{g}/\text{mg}$ dry wt.	8.9	11.2	8.2	7.8
Type of growth	—	Two-dimensional	One-dimensional	

The next experiment was arranged to show a reversibility of the inhibition preferentially induced on the two-dimensional growth by the analogs. It was found that the simultaneous addition of the same concentration of methionine or tryptophane respectively to ethionine or 5-methyltryptophane completely compensates the action of these analogs on the morphological differentiation. In these experiments simultaneous administration was done on the cultures of spore, protonema of five-cell stage, and young prothallium 10 days after the two-dimensional differentiation.

If methionine or tryptophane was added to the culture in which the "artificial" one-dimensional growth induced by amino acid analogs was going on, a complete reversal of the one-dimensional to the two-dimensional growth was observed. In such experiments the amino acid analogs were first added to the culture containing organisms at the developmental stages mentioned above, and after 14 days methionine or tryptophane was introduced, respectively.

### 3. Effect of 8-azaguanine

As it is generally accepted that nucleic acids are involved in the protein synthesis, the question may be raised whether the intense protein synthesis which accompanies the two-dimensional differentiation is connected with nucleic acids. 8-azaguanine is known to be the most effective inhibitor of synthesis of RNA. It was therefore designed to use this which would selectively suppress the RNA metabolism.

To find the optimum concentration of this substance which might selectively

affect the two-dimensional growth, concentrations from M/500 to M/25,000 were applied to the culture medium. Stages used in this test involved spores, the prothallium of five-cell stage, the young plate-like prothallium a few days after the two-dimensional differentiation, and 50 days old prothallium. At a concentration below M/17,500 no morphological effect was noted by 8-azaguanine. The substance above M/1,000 caused gradual or immediate death depending on the concentration applied. However in the concentration range between M/12,000 and M/14,000 a preferential inhibition of the two-dimensional growth was clearly observed. As the results obtained were comparable with those found in the case of amino acid analogs, they are not redescribed here.

The next experiment is made to show that the specific inhibitory effect of 8-azaguanine at the concentration of M/12,500 could be obtained at any developmental stage, 8-azaguanine being added to the culture of spore, protonema of five-cell stage, prothallium a few days after the two-dimensional differentiation and 50 days old prothallium; the effect of 8-azaguanine was examined on 20th day and 30th day after the addition. Here again, at each stage tested, upon addition of 8-azaguanine the two-dimensional growth was completely suppressed and converted into the one-dimensional, while the one-dimensional growth was not affected.

Alleviation of the growth inhibition by 8-azaguanine was achieved by the addition of the same concentration of guanine. When the guanine was added simultaneously with 8-azaguanine and tested on spore, protonema of five-cell stage, young prothallium a few days after the two-dimensional differentiation and 50 days old prothallium, no inhibitory effect on the two-dimensional growth was observed.

If guanine was added to the culture in which the "artificial" one-dimensional growth was taking place in the presence of 8-azaguanine, a complete reversal of 8-azaguanine effect became apparent immediately after the addition of guanine. In this series of experiments 8-azaguanine was first added to the culture containing five different stages of development and after 14 days the same amount of guanine was introduced. Morphological appearance of organisms in each culture ten days after guanine introduction is schematically illustrated in Fig. 2. The results mentioned above strongly suggest that 8-azaguanine inhibits the two-dimensional growth by interfering with the RNA metabolism. The fact that both amino acid analogs and nucleic acid antagonist cause the same inhibitory effect on the two-dimensional growth would indicate that the specific RNA synthesis sensitive to 8-azaguanine is necessary for the formation of protein characteristic for the two-dimensional growth, which is sensitive to the amino acid analogs. This being the case, it might be expected that the formation of protein detectable during the two-dimensional growth is completely inhibited by 8-azaguanine. To test this, 8-azaguanine was added at the concentration of M/12,500 in the culture containing spore, protonema of five-cell stage, and 50 days, 60 days and 80 days old prothallium. All cultures were allowed to develop for 5 to 15 days only in the one-dimensional growth pattern. In Fig. 2 the data of protein estimation of such cultures are compared with those of the corresponding controls in which no 8-azaguanine had been introduced. The results clearly demonstrate

that 8-azaguanine causes a decrease in protein concentration and simultaneously a complete inhibition of prothallium formation characteristic for the two-dimensional growth. The situation is exactly comparable to the case of amino acid analogs. Upon introducing guanine after five and fifteen days after 8-azaguanine addition, a marked increase of protein concentration was demonstrated with a simultaneous commencement of the two-dimensional growth. The results of this experiment are also included in Fig. 2.

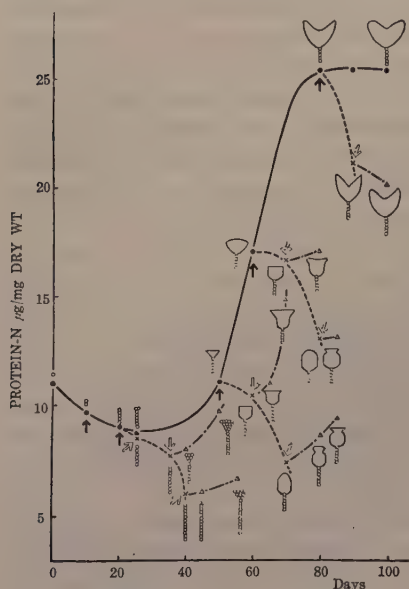


Fig. 2. Effect of 8-azaguanine on the differentiation and protein content of young prothallium of *Dryopteris erythrosora* with the reversal of 8-azaguanine effect by the addition of guanine. ●—●: protein-N per mg dry weight in control culture; ×—×: protein-N per mg dry weight in the cultures to which M/12500 8-azaguanine was added at the time indicated by black arrow; △—△: protein-N per mg dry weight in the cultures to which first 8-azaguanine was added, followed by the introduction of the same concentration of guanine at the time indicated by white arrow. Appearance of prothallium was schematically presented at each point.

nancy of another metabolic activity. On the other hand, two-dimensional growth is supported by a higher level of protein synthesis plus lower activity of another process. Thus the lowering of protein synthesis by 8-azaguanine and amino acid analogs caused the competitive increase in another activity. As a result, the mode of growth is converted from two-dimensional to one-dimensional.

Now, if the first explanation is correct, then it would follow that the interference of general metabolism such as respiration, phosphorylation, and glycolysis

#### 4. Effects of general metabolic inhibitors

The fact that the two-dimensional growth (and the accompanied protein synthesis) is completely inhibited by interfering either with protein synthesis or with the RNA metabolism is indicative of these processes being characteristic of the two-dimensional growth. There are at least three ways of interpreting those results:

a. Two-dimensional growth requires a certain level of protein synthesis which is qualitatively not different from that of one-dimensional growth. 8-azaguanine and amino acid analogs cause non-specific lowering of the synthetic rate of protein down to the level, thus resulting in the conversion of the mode of growth.

b. Two-dimensional growth is causally connected with the formation of a distinct protein(s) which is specifically inhibited by 8-azaguanine and amino acid analogs.

c. Two types of growth in the problem are preceded by a combination of two (or more) metabolic processes which are qualitatively common in both. One-dimensional growth is brought about by a lower level of protein synthesis and a predomi-



(which result from the lowering of the protein synthesis) may equally result in an inhibition of the two-dimensional growth without affecting the one-dimensional growth. On the other hand, if the second or third process is involved, the introduction of a general inhibitor would cause only non-specific interference of growth in its effective range of concentration without preferential inhibition of the two-dimensional growth.

In the first series of experiments, spores were allowed to germinate in the presence of inhibitors in a wide range of concentrations ( $10^{-2}$  to  $10^{-14}$ M). The inhibitors used were KCN,  $\text{NaN}_3$ , 2,4-dinitrophenol, NaF, and sodium malonate. Those inhibitors either caused a simple inhibition of growth, or were without effect. No inhibitor at any concentration tested could cause a selective inhibition of the two-dimensional growth, or a conversion from the two-dimensional to the one-dimensional growth.

The second series of experiments were carried out so that the inhibitors mentioned above were applied to the culture containing the young plate-like prothallium in the two-dimensional growth (seven days after inception of the two-dimensional growth). In no case was the preferential inhibition of the two-dimensional growth obtained: the inhibitors caused either death or a general retardation of growth depending on the concentration of inhibitors applied. In lower concentrations the growth and differentiation were not affected at all.

#### 5. Incorporation of $^{14}\text{C}$ -8-azaguanine into RNA

It has been shown that 8-azaguanine selectively suppresses two-dimensional growth, allowing one-dimensional growth to proceed. It is possible that 8-azaguanine actually incorporates into RNA to make abnormal RNA, which then causes the change of the growth type. The young prothallium 10 days after initiation of the two-dimensional growth was transferred into the culture medium containing  $^{14}\text{C}$ -8-azaguanine (M/12,500), and cultured for 30 days. Microscopic observation revealed that the growth pattern of nearly all prothallia had been converted from two-dimensional to one-dimensional. Fig. 3 shows that the radioactivity is sharply confined around the region of 8-azaguanic acid [6], although no detectable ultraviolet absorbing material was found there.

We could neither isolate nor identify 8-azaguanic acid, but the chromatographic position of radioactivity strongly suggests that 8-azaguanine which had incorporated

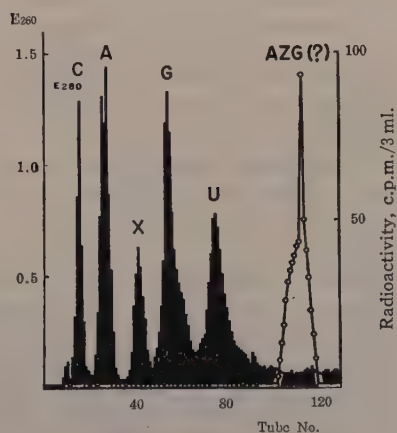


Fig. 3. Chromatographic separation of individual mononucleotides of RNA from the prothallium of the fern, *Dryopteris erythrosora*, cultured in the presence of  $^{14}\text{C}$ -8-azaguanine. Black bar: ultraviolet absorption; Open circle: radioactivity. C=Cytidylic acid, A=Adenylic acid, G=Guanylic acid, U=Uridylic acid, AZG=8-azaguanic acid, X=Unknown ultraviolet absorbing substance.



into RNA was recovered as 8-azaguanic acid after the alkaline hydrolysis of RNA. Assuming that the radioactivity on the chromatogram is due to 8-azaguanic acid, it was then calculated that 8-azaguanic acid content of the RNA was about 0.85 per cent of guanylic acid. No radioactivity was demonstrated in other ultraviolet absorbing substances on the chromatogram. It is clear from the chromatogram that the RNA formed in the presence of 8-azaguanine contains an ultraviolet absorbing compound (Fig. 3, "X") beside cytidylic, adenylic, guanylic, and probably 8-azaguanic acids. The nature of this substance is not known yet, but a preliminary analysis showed that it contained phosphorus.

#### 6. Changes in the RNA content

Several routine procedures of chemical determination of RNA had failed to

TABLE 2

Comparison of two different procedures of RNA hydrolysis for quantitative estimation for analysis of nucleotide composition

Sample: Cold-acid treated, lipid-free dry powder  
of fern prothallium 40 days after germination

Molar ratio (adenylic acid=10.0)

Mononucleotide mixture obtained by	Guanylic	Cytidylic	Uridylic	Purine Pyrimidine	RNA recovered (mg/g powder)
Alkaline hydrolysis <sup>1)</sup>	13.0	9.2	10.2	1.19	3.15
Ribonuclease digestion <sup>2)</sup>	12.6	9.2	10.1	1.17	3.21

1) With 0.5 N KOH at 37°C for 20 hours.

2) With 0.2 mg/ml ribonuclease at 37°C for 18 hours followed by alkaline hydrolysis with 0.1 N KOH at 80°C for 20 minutes.

estimate the reasonable value in the present material because of the presence of interfering substances. We then tried to estimate RNA content by the quantitative separation of mononucleotides on the column after the direct alkaline hydrolysis of RNA from the cold-acid treated, lipid-free tissue powder. However difficulty was sometimes encountered in the course of nucleotide separation on the column by the presence of interfering ultraviolet absorbing substances of brownish colour in the hydrolysate. It was finally found useful first to digest RNA in the tissue powder by ribonuclease, and then to hydrolyze with alkali. The chromatographic separation of mononucleotides with Dowex-1 [7] followed. Table 2 indicates that the amount of RNA in the tissue was quantitatively recovered by the procedure mentioned above, the recovery being comparable with that obtained by the direct alkaline hydrolysis of RNA plus subsequent fractionation of RNA mononucleotides. Essentially the same results were obtained by the same type of comparison done on rat liver RNA. We therefore used the ribonuclease technique throughout this study.

In the first place, changes in the RNA content during the formation of

gametophyte were followed. From Fig. 4, it is clear that the amount of RNA *per plant* slightly increased during the primary one-dimensional growth. Onset of two-dimensional growth was accompanied by a very rapid synthesis of RNA. About 60 days after germination, the RNA content began to decrease, and the decrease continued up to 80 days. In the following 20 days, the amount increased again. (This increase was probably due to the initiation of the sexual organ formation, and will not be discussed in this paper). Although these changes resemble those found in protein (Fig. 1), RNA began to increase somewhat earlier than protein, and it decreased later rapidly where protein still continued to increase. Changes in RNA concentration (RNA per dry weight) are illustrated in Fig. 4. The first slight drop in the concentration of RNA was followed by a rapid increase during the primary one-dimensional growth. In the course of two-dimensional growth, the concentration first increased, then decreased very slowly up to about 70 days, and finally fell sharply. Unlike the case of protein, primary one-dimensional growth was characterized by a sharp increase of RNA concentration. Furthermore, the two-dimensional growth of the later phase was accompanied by the decrease of RNA concentration. It is thus clear that the over-all concentration of RNA in the cell *per se* has no immediate correlation with the mode of growth.

In the next series of experiments, the changes in the RNA content during the artificial one-dimensional growth induced by 8-azaguanine (M/12,500) was measured. 8-azaguanine was added to the young prothallia 20 days after the onset of two-dimensional growth. Prothallia were harvested 20, 30 and 40 days after the introduction of the analog. The results included in Fig. 2 and Fig. 4 show that the increase of RNA was completely inhibited by this analog, and the amount of RNA greatly decreased in later stages. When 8-azaguanine was added to the old prothallium culture (35 days after the onset of two-dimensional growth) the amount of RNA decreased strikingly in the following 20 days. The concentration of RNA (per dry weight) was definitely decreased in both cases by the addition of 8-azaguanine. Above results were roughly comparable to those obtained in the case of protein under the similar experimental conditions.

#### 7. Relation of abnormal RNA to the mode of growth

It has been mentioned that RNA obtained from the prothallia cultured in

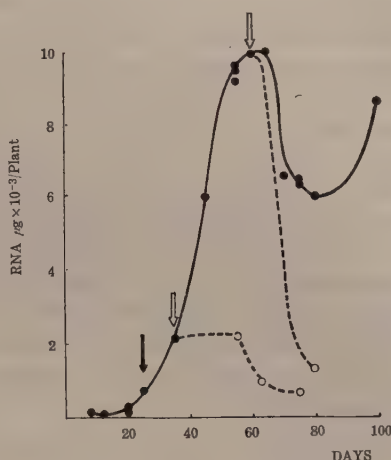


Fig. 4. Changes in the RNA content per single plant during the course of germination and prothallium formation in *Dryopteris erythrosora*. Black arrow: the beginning of the two-dimensional differentiation. White arrow: the time of 8-azaguanine addition. Solid circles: the RNA content in normal development; Open circles: the RNA content in the artificial one-dimensional growth induced by 8-azaguanine.

the presence of 8-azaguanine contained an unknown ultraviolet absorbing compound and in all likelihood a trace of 8-azaguanic acid. The RNA from all young normal prothallia at several stages of one-dimensional growth possessed neither substance "X" nor 8-azaguanic acid. Then, the appearance of "X" in RNA was traced after the addition of 8-azaguanine to see whether it reveals any chronological correspondence to the conversion of the growth pattern. As shown in Table 3, the presence of "X" could not be found in RNA from young prothallia cultured about 20 days in the presence of 8-azaguanine, although the mode of growth was definitely converted from two-dimensional to one-dimensional. The substance "X" appeared only when the young prothallium was cultured with 8-azaguanine for more than 27 days, or when the old prothallium was treated by the analog for 20 days. These results appear to preclude a possible involvement of "X" in the selective inhibition of two-dimensional growth, although it is possible that a trace of this substance in RNA could play a decisive role. It may be tentatively concluded that 8-azaguanine suppresses the formation of RNA, or inactivates it, probably by incorporating into RNA, but not by forming "X" in RNA.

#### 8. Changes in nucleotide composition of RNA

We have speculated that the two-dimensional growth would be brought about by the synthesis of protein(s) characteristic of this mode of growth. The results discussed in the foregoing sections strongly suggest that RNA is somehow involved in determination of the mode of growth. As the theory of involvement of RNA in the synthesis of protein has been widely accepted, the present results

might be taken to mean that RNA controls the mode of growth via metabolism of protein. If the qualitative difference of RNA reflects the specificity of protein, then it may be supposed that each type of growth is accompanied by the presence of specific RNA.

Keeping the above discussion in mind, the nucleotide composition of *total* RNA during primary one-dimensional, two-dimensional, and artificial one-dimensional growth was followed. Mononucleotide solution obtained by the direct alkaline hydrolysis of the acid-treated, lipidfree tissue powder was very often contaminated by substances of deep brownish red colour, which strongly interfere with the chromatographic separation of nucleotides with Dowex-1. We have therefore adopted the same technique as was used in the quanti-

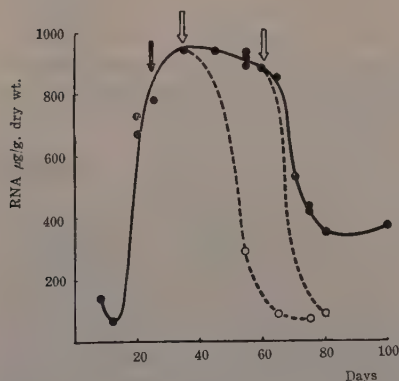


Fig. 5. Changes in RNA concentration ( $\mu\text{g/g}$  dry matter) during the course of germination and the formation of prothallium in *Dryopteris erythrosora*. For symbols, etc., see legend of Fig. 4.

tative estimation of RNA (details, see MATERIAL AND METHOD). The nucleotide composition determined by the present technique using ribonuclease has been



compared with that determined by our standard procedure described previously [7], involving direct 0.5 N KOH hydrolysis of RNA in tissue powder at 37°C for 20 hours. As shown in Table 2, there is no essential difference between the values obtained by the two techniques. A similar comparison made on the samples of rat liver again indicated no definite difference. The results of nucleotide analysis of RNA from different phase of gametophyte development are shown in Table 3. RNA obtained from the prothallium in the course of one-dimensional growth was

TABLE 3

Average nucleotide composition of RNA of the fern prothallium at different stages of primary one-dimensional, two-dimensional, and artificial one-dimensional growth  
Molar ratio (Adenylic=10.0)

Source of RNA	Mode of growth	Guanylic	Cytidylic	Uridylic	Purine Pyrimidine
One cell stage	Primary one-dimensional	15.6	7.4	12.9	1.3
Two cell stage	"	lost	7.2	12.5	—
Five cell stage	"	12.2	6.8	11.8	1.2
Five cell stage	"	13.0	6.7	12.1	1.1
Seven cell stage	"	13.1	8.1	12.2	1.1
35 days old prothallium	Two-dimensional	12.5	10.1	10.0	1.1
35 days old prothallium	"	12.4	10.3	9.4	1.1
45 days old prothallium	"	12.6	10.6	9.6	1.1
55 days old prothallium	"	13.1	10.1	10.4	1.1
55 days old prothallium	"	12.7	10.6	9.6	1.1
60 days old prothallium	"	13.3	11.0	8.6	1.2
70 days old prothallium	"	13.5	10.5	8.6	1.2
75 days old prothallium	Two-dimensional	12.9	10.7	8.6	1.2
8-azaguanine treated prothallium <sup>1)</sup>	Artificial one-dimensional	13.1	8.1	12.2	1.1
8-azaguanine treated prothallium <sup>1)</sup>	"	14.0	8.3	11.9	1.2
8-azaguanine treated prothallium <sup>2)</sup>	"	10.8	7.7	13.3	— <sup>4)</sup>
8-azaguanine treated prothallium <sup>2)</sup>	"	9.9	8.5	13.9	— <sup>4)</sup>
8-azaguanine treated prothallium <sup>3)</sup>	"	9.7	9.3	11.7	— <sup>4)</sup>

1) 20 days' treatment with 8-azaguanine (M/12,500) from 10th day after onset of two-dimensional growth.

2) 27 days' treatment with 8-azaguanine (M/12,500) from 10th day after onset of two-dimensional growth.

3) 40 days' treatment with 8-azaguanine (M/12,500) from 10th day after onset of two-dimensional growth.

4) "X" present.

characterized by higher contents of guanylic acid and uridylic acid as compared with contents of adenylic acid and cytidylic acids. The RNA is tentatively designated "U-type". This relationship held true in all RNA samples from all stages of primary one-dimensional growth studied. On the other hand, the RNA from the prothallium in the two-dimensional growth phase had higher contents of guanylic acid, lower contents of cytidylic acid, and intermediate contents of adenylic acid and uridylic acid ("C-type"). No remarkable changes of nucleotide composition was observed during the two-dimensional growth. These results clearly indicate differences in average nucleotide composition of RNA from prothallia in different types of growth.

In the next place, the changes in nucleotide composition of RNA during artificial one-dimensional growth produced by 8-azaguanine was investigated (Table 3). 20 days after the onset of two-dimensional growth, 8-azaguanine (M/12,500) was introduced into the culture medium, and the cultivation was continued for 20, 27 and 40 days. The results indicated that RNA obtained from the prothallia 20 days after the addition of the analog reveals U-type nucleotide composition. Also the RNA obtained from the prothallia 27 and 40 days after the addition of the analog showed a nucleotide composition similar to that of 20 days' sample. However, it is obvious from Table 3 that the RNA had a lower guanylic acid content as compared with other RNA mentioned above, and further it contained the fifth ultraviolet absorbing material "X". The impression was obtained that the amount of "X" compensated the difference in the content of guanylic acid. Although we have not yet succeeded in identifying this component, this RNA could be considered to be of U-type, if "X" was derived from guanylic acid.

TABLE 4  
Average of all data in Table 3  
Molar Ratio (Adenylic acid=10.0)

Source of RNA: Prothallium in	Guanylic	Cytidylic	Uridylic
Primary one-dimensional growth	13.5	7.2	12.3
Two-dimensional growth	12.9	10.5	9.2
Artificial one-dimensional growth*	13.5	8.2	12.0

\* Data of RNA having "X" were omitted.

The above set of facts indicates that the one-dimensional growth, whether it is normal or artificial, is correlated with the existence of U-type RNA, and two-dimensional growth is characterized by the presence of C-type RNA (see Table 4).

## DISCUSSION

The present studies suggested that the two-dimensional growth was causally connected with the rapid synthesis of protein. Whether the protein synthesized at the time of the two-dimensional growth is of a distinctly specific nature or

not must be left open for the time being. However, the fact that a general inhibition of the cellular metabolism does not cause selective inhibition appears rather to favor an involvement of specific protein formation which is selectively affected by amino acid analogs or 8-azaguanine.

As for the mode of action of those inhibitors, we do not have direct evidence. One possibility is that the formation of RNA is indispensable as a component of protein synthesizing machinery, and that 8-azaguanine incorporates into RNA moiety of this machinery in place of guanine residue, rendering it inert.

Amino acid analogs might be directly incorporated in the postulated specific protein as its synthesis occurs, so that the two-dimensional growth is inhibited. However it is equally possible to assume that analogs incorporate into the protein synthesizing machinery, and make it abnormal.

At any rate, the fact that 8-azaguanine specifically inhibits the two-dimensional growth with a simultaneous stop of protein synthesis immediately points to the possibility that the metabolism relating to RNA is one of the essential processes of the two-dimensional growth. It was demonstrated that during conversion of the growth type from two-dimensional to one-dimensional, an incorporation of 8-azaguanine into RNA was observed with a concomitant stop of increase of RNA, or its degradation. Although the incorporation was often accompanied by the appearance of an unknown ultraviolet absorbing substance "X" in RNA, the substance does not seem to be the cause of inhibition of either two-dimensional growth or the synthesis of RNA. The presence of this substance could not be demonstrated in RNA from the young prothallium cultured for a short time with 8-azaguanine, though the mode of growth was converted from two-dimensional to one-dimensional. The "X" would thus represent the degradation process of RNA related to the pathological conditions of the organism. The primary cause of the conversion of the growth pattern might be the synthesis of abnormal RNA having 8-azaguanilyc acid, which inhibits a subsequent synthesis of RNA. The behaviour of RNA just discussed above would be interpreted by assuming that the synthesis of RNA or the presence of a certain amount of RNA in the cell is necessary for the two-dimensional growth. However, the situation is not as simple as it would appear, because the young prothallium in the primary one-dimensional growth rapidly synthesizes RNA and has a high RNA content. Moreover, in the later stages of two-dimensional growth, the increase in the amount of RNA stops, and the degradation of RNA follows. These results might indicate that a preliminary accumulation of RNA is a prerequisite for the initiation of two-dimensional growth.

A more attractive hypothesis which is compatible with the facts observed would be that the two-dimensional growth is supported by a special type of RNA which is extremely susceptible to the action of 8-azaguanine.

The data of average nucleotide composition of RNA clearly indicated that each type of growth is characterized by the RNA having a distinct composition. It was moreover shown that the RNA found in the artificial one-dimensional prothallium produced by 8-azaguanine had practically the same composition as that of primary one-dimensional growth. We do not know whether the difference



of the average nucleotide composition reflects the presence of different molecular species of RNA, or mere changes in some nucleotide contents in certain part of the same RNA. The intermolecular heterogeneity of RNA depending upon the intracellular localization [2, 3, 4, 7] rather suggests the former possibility. A simple explanation of the results might be that the U-type RNA is responsible for the one-dimensional growth, and the C-type RNA for the two-dimensional growth. It is, however, not likely that the U-type RNA completely disappears by initiation of the two-dimensional growth and is resynthesized *de novo* by the action of 8-azaguanine. More plausible would be that what we called C-type RNA is a mixture of predominant amount of C' RNA plus smaller amount of U' RNA. Similarly, it is possible that the U-type RNA is a mixture of a trace of C' RNA and much larger amount of U' RNA. The two-dimensional growth would require a certain amount of C' RNA which is seriously affected by 8-azaguanine.

### SUMMARY

Estimation of the protein contents during the course of development clearly indicates that whenever the two-dimensional growth takes place, a sharp increase in protein concentration in the cell can be observed, whereas in the course of the primary one-dimensional growth, protein concentration is gradually lowered. It has been shown that the two-dimensional growth can be specifically inhibited by some amino acid analogs such as ethionine and 5-methyltryptophane and it is converted to the "artificial" one-dimensional growth. The same selective interference of the two-dimensional growth is also brought about by 8-azaguanine, the effective inhibitor of the nucleic acid synthesis. Further it has been demonstrated that such inhibition can be alleviated by adding corresponding amino acids or guanine, respectively.

Regardless of the nature of the effective inhibitors used, the selective inhibition is always accompanied by an immediate cessation of increase in the protein concentration, followed by its gradual decrease.

It has been found that in parallel to the inhibition with 8-azaguanine of two-dimensional growth, the analog was found to incorporate into RNA, when tested with  $^{14}\text{C}$ -8-azaguanine.

The study of changes in the RNA content during the gametophyte development has demonstrated no simple correlation of over-all concentration of RNA or of the rate of increase of RNA to the mode of growth. Conversion of the two-dimensional growth to the one-dimensional growth by the action of 8-azaguanine is followed by the stop of increase in the RNA content or by degradation of RNA.

Finally, it has been shown that RNA from the prothallium in the two-dimensional growth possesses a nucleotide composition distinct from that in the one-dimensional growth. RNA found in the prothallium in the one-dimensional growth induced artificially by 8-azaguanine has been shown to have practically the same nucleotide composition as that of the primary one-dimensional growth.

From these observations, it has been tentatively inferred that a special RNA

fraction which is sensitive to the action of 8-azaguanine is closely related to the two-dimensional growth.

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## PAPER CHROMATOGRAPHIC SURVEY OF ANTHOCYANIN IN TULIP-FLOWERS, I.<sup>1)</sup>

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In the preceding papers of this series of study on the anthocyanin pigments of tulip-flowers, the senior author (M.S.) isolated them successfully in crystalline state and described precisely their properties and structure [16, 17]. But the procedures were all time-consuming, involving treatment of a large amount of samples. It is desirable to know all the kinds of the flower pigments, anthocyanins in this case, of a variety using one or a few flowers. So we have carried out chromatographically the detailed examination of anthocyanin components of the flowers of many garden varieties of tulip.

### MATERIALS AND METHODS

In the present study the flower of one hundred and seven garden varieties of tulip were examined. They had been imported to Japan since 1910 from Holland and preserved in the garden of the Tonami Horticultural Branch, Toyama Prefectural Agricultural Experiment Station.

The name of strains and garden varieties used were shown in Table 1.

The experiments were chiefly carried out according to BATE-SMITH's technique [3] improved by HAYASHI [6], HAYASHI et al. [7] and ABE et al. [1]. The samples were developed throughout this study by the unidimensional ascending procedure on Tôyô No. 52 filter paper (40×40 cm) at  $28\pm1^{\circ}\text{C}$  in the thermostat.

The developing solvents employed were tabulated as Table 2.

#### 1. *Collection of materials and extraction of anthocyanin*

Early in May, 1958, one flower for each garden variety of tulip was collected from the garden of the Tonami Horticultural Branch, Toyama Prefectural Agricultural Experiment Station and treated immediately.

For the preparation of anthocyanin mixtures available for the chromatographic analysis, the fresh perianth was immersed in 20 ml. of cold methanol containing 1 per cent hydrochloric acid over-night and filtered. The filtrate containing extracted pigments was concentrated in a desiccator at laboratory temperature and then stored in the refrigerator.

#### 2. *Preparation of anthocyanins*

Each of the pigments in the concentrated filtrate isolated by means of mass

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2) Biological Institute, Toyama University, Toyama.

3) In testimony of my friendship and high regard for Professor TADAO JIMBO as teacher, investigator and editor, I wish to dedicate this paper to him on the occasion of his sixty-third birthday.



TABLE 1. The name of strains and garden varieties examined

Strain	Garden Variety
Darwin	Adajio, Charles Needham, Demeter, Desirée Overall, Fridtof Nansen, Faust, Feu Brilliant, Greuze, Hyrack Brum, King Maure, La Tulipe Noire, Lucifer, Merveille de Haarlem, Mr. Van Zijl, Mrs. Potter Palmer, New Orleans, Margaux, Paul Richter, Phillippe de Comines, Pride of Haarlem, Prince of Wales, Professor Rauwenhof, Queen of Hearts, Queen of Night, Red Master, Red Queen, Reverand Ewbank, Rose Copland, Royal Present, Scarlet O'Hara, Scarlet Sensation, Scotch Lassie, Sieraad van Flora, Suzon, The Bishop, The Sultan, Tindal, Utopia, Valentine, Victoire d'oliveira, Viola.
Early Single	Brilliant Star, Brilliant Star Maxima, Couleur Cardinal, Cramoisie Brilliant, Joost van den Vondel, Pottebakker Scarlet, President Lincoln, Prince of Austria, Purple Crown, Van der Neer, Variegation Cochenille.
Early Double	Aurora, Cochenille, Dante, Electra, Paul Crampel, Titian, Triumphator, Vuurbaak.
Late Double	Orange Triumph, Symphonia.
Mendel	Fuga, Hildegarda, John Gay, Jujirihem, Krelage Triumph, Olaf, Orange Wonder, Poussin, Topscore, Zenober, Van der Eerden.
Triumph	Alberio, Aviateur, Bandoeny, Crown Imperial, Delden, Johanna, Korneforos, Marjorie, Mississippi, Orange Burcht, Princess Beatrix, Telescopium.
Cottage	Advance, Amaranthe, Barbara Pratt, Caledonia, Cocarde, Geisha, Majestic, Marshal Haig.
Parrot	Allard Pierson, Black Parrot, Blue Parrot, Parrot Pierson, Therése.
Breeder	Jan van Galen, Prince of Orange, Valcain, Velvet King.
Tulipa	Eichleri Excelsa, Red Emperor.
Lily Flowering	Captain Fryatt.

TABLE 2. Abbreviations and compositions of developing solvents used

Abbreviated designation	Composition of developing solvent and its ratio (v/v)	Applied for
Bu. H	n-buthanol/conc. hydrochloric acid/water (7:2:5)	anthocyanin
AA. H	Acetic acid/conc. hydrochloric acid/water (5:1:5)	anthocyanidin
Bu. Amph	n-buthanol saturated with 0.5 N (NH <sub>4</sub> ) <sub>2</sub> HPO <sub>4</sub>	organic acid
Bu. AA-1	n-buthanol/acetic acid/water (4:1:2)	sugar
Bu. AA-2	n-buthanol/acetic acid/water (4:1:5)	sugar
Bu. Pyrd	n-buthanol/pyridine/water (6:3:1)	sugar

paper-chromatography (unidimensional ascending procedure) at laboratory temperature, appeared on the filter paper in separate bands—several colour bands of anthocyanin. Then the proportion of the pigments was estimated. Each colour band was separated by cutting and then eluted by unidimensional descending procedure in methanol containing 5 per cent acetic acid. The eluate was imme-

diately developed by Bu.H and its Rf-value was measured. The authentic specimens of tulipanin, chrysanthemin, keracyanin and pelargonin were also co-chromatographed.

### 3. *Detection of the bound organic acids*

Acylated anthocyanins were sometimes found in fruits, leaves and flowers of plants [5, 9, 10, 11, 12, 18].

To detect organic acids, the purified anthocyanin eluate (cf. 2) was treated as follows: 5 ml. of the eluate of each anthocyanin was treated for 1 hour with equal volume of 20 per cent sodium hydroxide solution in an atmosphere of hydrogen. After the solution was acidified with conc. hydrochloric acid, the free organic acids were then extracted repeatedly with diethyl ether. The combined diethyl etherial solution was evaporated to dryness. The residues and the authentic malonic acid and p-hydroxybenzoic acid were co-chromatographed by using Bu.Amph as developing solvent. Over the chromatogram was sprayed ethanol containing 0.02 per cent methyl red for detection of organic acids.

### 4. *Hydrolysis of anthocyanin*

Each anthocyanin solution (ca. 5 ml.) was boiled with 5 ml. of 20 per cent hydrochloric acid for 3-5 min., chilled in the refrigerator, and after addition of water, the resultant aglycone was extracted with a small quantity of iso-amyl alcohol and chromatographed.

The hypophasic aqueous layer was used for the determination of sugars.

#### a. *Anthocyanidins*

The iso-amyl alcoholic layer was dirty dark red-brown in colour, so 2 volumes of water and 4 volumes of benzene were added and shaken. The anthocyanidin which had gone into the watery layer was extracted repeatedly with isoamyl alcohol and then applied for chromatographic test with AA.H as developing solvent.

With it were co-chromatographed the authentic specimens of delphinidin from tulipanin, cyanidin from keracyanin and chrysanthemin and pelargonidin from pelargonin.

#### b. *Sugars*

The hypophasic aqueous solution containing hydrolytic decomposition products of each anthocyanin was dried under reduced pressure in the sodium hydroxide-containing desiccator and dissolved in a small quantity of water and co-chromatographed by the use of Bu.AA-1, Bu.AA-2 or Bu.Pyrd as developing solvent with the authentic specimens of rhamnose, xylose, glucose, galactose and arabinose, the only known sugar components attached to the anthocyanin molecule.

For detection of sugars the following reagents were sprayed over the chromatograms: ethanolic solution of 1 per cent aniline hydrochloride, 2 per cent anisidine hydrochloride, 5 per cent benzidine-acetic acid, 0.1 per cent potassium permanganate solution, aniline-phthalic acid and ammoniacal silver nitrate solution.

## RESULTS

1. *Determination of anthocyanin*

From the present chromatographic study, it was found that at least six kinds of anthocyanin were present in the flowers of the tulip varieties and each variety generally contains 2-4 kinds of anthocyanin except a garden variety "Telescopium" which contains only one kind of anthocyanin. These spots of the anthocyanins were designated tentatively as A<sub>1</sub>, A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>, A<sub>5</sub> and A<sub>6</sub>, respectively, and their Rf-values were as follows:

TABLE 3. The Rf-values of isolated anthocyanins in Bu. H.

Designation	A <sub>1</sub>	A <sub>2</sub>	A <sub>3</sub>	A <sub>4</sub>	A <sub>5</sub>	A <sub>6</sub>
Rf-value	0.62-0.66	0.50-0.54	0.41-0.44	0.36-0.39	0.26	0.18-0.19

2. *Determination of anthocyanidins (aglycones)*

After hydrolytic decomposition of the anthocyanins, the corresponding anthocyanidins were carefully compared with each other on the chromatogram (Table 4).

TABLE 4. Rf-values of authentic anthocyanidins and the pigments from tulip-flowers

Authentic anthocyanidins	Rf-values in AA.H	Samples from	Rf-values in AA.H
Delphinidin	0.25	A <sub>4</sub> , A <sub>5</sub> , A <sub>6</sub>	0.23-0.25
Cyanidin	0.39	A <sub>2</sub> , A <sub>3</sub>	0.37-0.41
Pelargonidin	0.57	A <sub>1</sub>	0.55-0.59

These results indicate that A<sub>1</sub> belongs to the glucoside of pelargonidin, A<sub>2</sub> and A<sub>3</sub> to that of cyanidin and A<sub>4</sub>, A<sub>5</sub> and A<sub>6</sub> to that of delphinidin. Generally speaking, the glucoside of cyanidin appears most frequently, that of pelargonidin less frequently and that of delphinidin least frequently.

3. *Determination of the organic acids and the sugars*a. *Organic acids*

The authentic specimens of p-hydroxybenzoic acid and malonic acid were used in chromatographic evaluation of the components of the anthocyanins in question. But the results of the experiment revealed that there were no bound organic acids in the anthocyanin molecule of tulips.

b. *Bound sugars*

From the results of the experiments, the sugar components were identified with glucose and rhamnose. The Rf-values of sugars contained in spots A<sub>1</sub>-A<sub>6</sub> and those of the authentic sugars are shown in Table 5.

4. *Preliminary identification of anthocyanins*

It was found so far that at least six kinds of anthocyanin were contained in the flowers of tulips.



TABLE 5. The relationship between the developing solvents and the Rf-values of the authentic sugars and the sugars in hydrolysate of tulip-anthocyanins

Authentic specimens \ Developing solvents	Rf-values in						
	Bu.AA-1	Bu.AA-2	Bu.Pyrd	Samples from	Bu.AA-1	Bu.AA-2	Bu.Pyrd
Galactose	0.25	0.21	0.22	A <sub>1</sub> , A <sub>2</sub> , A <sub>3</sub>	—	—	—
				A <sub>4</sub> , A <sub>5</sub> , A <sub>6</sub>			
Glucose	0.27	0.22	0.27	"	0.26-0.28	0.21-0.22	0.26-0.28
Arabinose	0.31	0.27	0.33	"	—	—	—
Xylose	0.34	0.31	0.39	"	—	—	—
Rhamnose	0.44	0.42	0.51	"	0.43-0.46	0.40-0.43	0.50-0.53

A<sub>1</sub> (Rf 0.62-0.66 in Bu.H) was not likely to be pelargonin (pelargonidin-3,5-diglucoside), for the Rf-value of pelargonin in Bu.H was 0.42. So far as the Rfvalue is concerned, it was rather similar to callistephin (pelargonidin-3-monoglucoside, Rf 0.60, [1]), but it was a diglucoside containing one molecule of glucose and rhamnose, respectively. If the sugars were attached at the 3- and 5-positions, the Rf-value of this anthocyanin probably would be similar to that of pelargonin. It appeared, therefore, likely to be pelargonidin-3-glucorhamnoside. Recently ASEN [2] identified one of the four anthocyanins in bracts of poinsettia with pelargonidin-3-glucorhamnoside by chromatographic and spectrophotometric methods. But pelargonidin-3-glucorhamnoside has not been hitherto obtained in a crystalline state. It may be interesting to crystallize this pigment and study its exact nature. In our laboratory it is now in progress.

A<sub>2</sub> (Rf 0.50-0.54 in Bu.H) was identified with keracyanin (cyanidin-3-glucorhamnoside), which was obtained in crystalline state by one of us from garden variety "Eclipse" of tulip [17].

A<sub>3</sub> (Rf 0.41-0.44 in Bu.H) was found to consist of cyanidin and glucose, so it must be either chrysanthemin (cyanidin-3-monoglucoside) or cyanin (cyanidin-3, 5-diglucoside). As the Rf-value of authentic chrysanthemin in Bu.H was 0.45 and that of cyanin in Bu.H was 0.28 [1] we can safely say that it is chrysanthemin.

A<sub>4</sub> (Rf 0.36-0.39 in Bu.H) was identified with tulipanin (delphinidin-3-glucorhamnoside), which was also crystallized by one of us from garden variety "Queen of Night" of tulip [16].

A<sub>5</sub> (Rf 0.26 in Bu.H) was identified as delphinidin-3-monoglucoside. KARRER et al. [8] reported the separation of slightly impure delphinidin-3-monoglucoside from the mixture of pigment present in *Viola tricolor* and its presence in bracts of *Hydrangea macrophylla* and autumnal leaves of *Rhus Toxicodendron* was also reported [13]. It was synthesized by REYNOLDS and ROBINSON [14] and the Rf-value of synthesized samples sent by ROBINSON was determined as 0.27 in Bu.H [6] (cf. Table 6).

A<sub>6</sub> (Rf 0.18-0.19 in Bu.H) was identified as delphin (delphinidin-3, 5-diglucoside). Delphin was isolated in crystalline state from the bright blue flower of *Salvia patens* CAV. in 1934 [15] and its Rf-value was determined as 0.16 in Bu.H

[6] (cf. Table 6), and 0.18 in *n*-butanol/conc. hydrochloric acid/water (5:1:2) at 25°C [4].

TABLE 6. Rf-values of authentic anthocyanins

Authentic specimens	Rf-value in Bu.H	Rf-value in Bu.H*	Remark
Pelargonin	0.42	0.42	* These figures were obtained by using Tôyô No. 50 filter paper at 25 ± 2°C. (HAYASHI, 1957)
Cyanin	—	0.28	
Chrysanthemin	0.45	0.43	
Keracyanin	0.53	0.54	
Tulipanin	0.39	0.38	
Delphin	—	0.16	
Delphinidin-3-monoglucoside	—	0.27	

From these facts and figures the six anthocyanins found in flowers of tulip might be formulated as follows:

- A<sub>1</sub>: Pelargonidin+glucose+rhamnose (unnamed, pelargonidin-3-glucorhamnoside ?).  
 A<sub>2</sub>: Cyanidin+glucose+rhamnose (keracyanin, cyanidin-3-glucorhamnoside)  
 A<sub>3</sub>: Cyanidin+glucose (chrysanthemin, cyanidin-3-monoglucoside)  
 A<sub>4</sub>: Delphinidin+glucose+rhamnose (tulipanin, delphinidin-3-glucorhamnoside)  
 A<sub>5</sub>: Delphinidin+glucose (unnamed, delphinidin-3-monoglucoside)  
 A<sub>6</sub>: Delphinidin+2 mol. glucose (delphin, delphinidin-3, 5-diglucoside)

##### 5. Relationship between anthocyanins and flower colours

The garden varieties of tulip which contain anthocyanins were classified roughly in the following 16 groups according to their flower colours (Table 7).

The results of the experiment were tabulated in Table 8:

By "delphinidin type" the authors mean a variety in which flower delphinidin occupies over fifty per cent of its total anthocyanidin. Similarly by "cyanidin type" and "pelargonidin type". Generally, garden varieties having black, black-purple, fade-sky, violet and purple flower belong to the delphinidin type, those having red-purple, pink, black-red, deep-crimson, crimson, deep-red, dark red-orange and red-orange flower to the cyanidin type and those having orange and flesh pink flower to the pelargonidin type. The figures of Table 8 also show that the cyanidin type occupies 52 per cent, pelargonidin type 27 per cent, and delphinidin type 12 per cent of the total tulip varieties examined.

## SUMMARY

Flower pigments, anthocyanins in this case, of one hundred and seven garden varieties of tulip (105 garden varieties of *Tulipa Gesneriana*, one garden variety of *T. Fosteriana* and one garden variety of *T. Eichleri*, respectively) were chro-

TABLE 7  
Classification of tulip varieties according to their flower colours

Flower colours	Garden varieties
Black	Black Parrot, Faust, La Tulipe Noire, Queen of Night, The Sultan.
Black-purple	Blue Parrot, Demeter, Dorrie Overall, Greuze, Hyrack Brum, Valcain, Valentine, Velvet King, Viola.
Fade-sky	Reverend Ewbank.
Violet	The Bishop, President Lincoln, Van der Neer.
Purple	Mrs. Potter Palmer, Scotch Lassie, Sieraad van Flora.
Red-purple	Electra, Jan van Galen, Margaux, New Orleans, Telescopium.
Pink	Barbara Pratt, Mr. van Zijl, Queen of Hearts, Rose Copland, Suzon, Symphonia.
Black-red	Allard Pierson, Geisha, Jujirohem, Parrot Pierson, Philippe de Comines.
Deep-crimson	Captain Fryatt, Couleur Cardinal, Majorie, Olaf, Purple Crown, Red Master, Scarlet Sensation, Zenober.
Crimson	Brilliant Star Maxima, Joost van der Vondel, Marshal Haig, Pottebakker Scarlet, Scarlet O'Hara, Vuurbaak.
Deep-red	Adajio, Dante, Fridtof Nansen, Fuga, Paul Crampel, Tindal, Victoire d'oliveira.
Red	Advance, Alberio, Amaranthe, Aurora, Aviateur, Brilliant Star, Charles Needham, Cocarde, Cochenille, Cramosie Brilliant, Crown Imperial, Desirée, Delden, Eichleri Excersa, Feu Brilliant, Hildegarda, Johanna, King Maure, Korneforos, Krelage, Majestic, Merveille de Haarlem, Mississippi, Paul Richter, Poussin, Pride of Haarlem, Prince of Austria, Prince of Wales, Princess of Beatrix, Professor Rauwenhof, Orange Burcht, Red Emperor, Red Queen, Royal Present, Thérèse, Titian, Topscore, Triumphator, Utopia, Van der Eerden, Variegation Cochenille.
Dark red-orange	Bandoeng, John Gay, Prince of Orange.
Red-orange	Lucifer, Orange Triumph, Orange Wonder.
Orange	Caledonia
Flesh-pink	Prunus.



TABLE 8. The relationship between flower colours and its anthocyanidins

Flower colour	Number of garden varieties examined	Number of delphinidin type	% of delphinidin	Number of cyanidin type	% of cyanidin	Number of pelargonidin type	% of pelargonidin
Black	5	1	50	—	29	—	21
Black-purple	9	7	65	1	30	—	5
Fade-sky	1	1	100	—	—	—	—
Violet	3	2	50	—	50	—	trace
Purple	3	2	43	1	50	—	7
Red-purple	5	—	12	3	66	—	22
Pink	6	—	2	4	65	2	33
Black-red	6	—	15	2	47	—	38
Deep-crimson	8	—	10	5	54	2	36
Crimson	6	—	2	4	55	2	43
Deep-red	7	—	3	5	51	2	46
Red	40	—	2	25	52	18	46
Dark red-orange	3	—	10	3	62	0	28
Red-orange	3	—	7	3	57	1	36
Orange	1	—	10	—	40	1	50
Flesh pink	1	—	trace	—	30	1	70

matographically investigated. They were separated from each other by mass paper-chromatography in comparatively pure state. Their components, i.e. anthocyanidin, sugar and organic acid which were decomposed by hydrolysis with acid and saponification with alkali, were studied also chromatographically.

The flower colours of tulips ranging from red to dark purple were caused by at least six kinds of anthocyanin and each garden variety generally contained 2-4 kinds of anthocyanin except a garden variety "Telescopium" which contained only one kind of anthocyanin. The results showed that the major pigment was tulipanin (delphinidin-3-glucorhamnoside) in purplish varieties, keracyanin (cyanidin-3-glucorhamnoside) in dark red and reddish varieties and unnamed pigment (pelargonidin-3-glucorhamnoside?) in orange red varieties. As minor pigments there were chrysanthemin (cyanidin-3-monoglucoside), delphin (delphinidin-3, 5-diglucoside) and unnamed delphinidin-3-monoglucoside in nearly all varieties. Although it is evident that flavonoids other than anthocyanin, and carotenoids etc. also play a role in flower colours, the colours of tulip-flowers are caused chiefly by combination of the above described six kinds of anthocyanin in various proportions. Characteristically, anthocyanins contained in tulips existed chiefly in the form of diglucoside, mostly glucorhamnoside, though they differ in respect of aglycone contained in them.

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# ON THE GROWTH OF PINE YEARLINGS IN COASTAL DUNE REGIONS WITH SPECIAL REFERENCE TO THEIR DROUGHT RESISTANCE

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## INTRODUCTION

Apart from intrinsic metabolism such as photosynthesis, respiration and nitrogen assimilation, water metabolism as a whole belongs to a passive phenomenon except for some active functions like root pressure, in other words, those physical functions which different from complex enzyme reactions come about spontaneously in plants provided with leaf-stem-root system. Nevertheless, the shortage of water in plants is immediately followed by mortality, and death by drying, in most cases, happens earlier than those by the deficiency of any other nutrients. So, water must be considered not only to be one of the most cardinal environmental factors but also to be an important inner factor for other intrinsic metabolism; water physiology is, consequently, tinged inevitably with ecological colouring. For ecological studies of metabolism, i.e., quantitative investigations of the relation between environmental factors and plant growth, the centremost metabolism will be matter production or the production of carbon containing substances as was ingeniously pointed out by Boysen Jensen as early as 1932 [1]. For the studies of ecological aspect of water relations in plants, therefore, we must not in principle lose sight of the bearing on matter production system. But the way to withering often proceeds with such a rapidity with the exhaustion of soil water that the water relations can be investigated separately in ecological studies of drought resistance.

There has been a vast number of papers on purely physiological studies of transpiration, water absorption, water conduction and so on, but studies on the synthetic aspect of water economy have been comparatively meagre and the reason for this will be as Walter [32] pointed out in his book that "Der Physiologe nur Teilvorgänge im Leben der Pflanzen analysiert. Er beschäftigt sich mit der Wasseraufnahme, der Wasserleitung und Wasserabgabe, nicht aber mit dem Wasserhaushalt als Ganzes". Montfort [13] discussed water economy in terms of "Bilanzquotient", i.e., the ratio of transpiration to water absorption and Huber [4] expressed mathematically this coefficient by several measures related to water economy. Later, Maximov [10] emphasized water balance in relation to drought resistance. As a component of water economy the amount of transpiration and water content of leaves were measured in various plants by several investigators, e.g., Monsi [12] in some wild and cultivated plants of Japan, Walter [31] in plants of Amani, East Africa and Pisek and Cartellieri [17] in plants of Europe. The determination of water absorption in

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normal and drought condition for land plants seems to be the weak point for the study of water economy, as water absorption can only be measured in such unnatural conditions as water culture and autoirrigated plants. So we are obliged to approach the problem of water absorption, especially in the shortage of soil water, from the availability of soil water. Since the establishment by Briggs and Shantz [2] of wilting coefficient or wilting percentage, studies on the availability of soil water have been promoted by several botanists and soil scientists among whom Veihmeyer [29] and Kramer [8] rendered many contributions to this problem. For the study of drought resistance the name of Stocker [20] [21] can never be missed who formulated different kinds of drought resistance. Drought resistance is usually defined as the nature of plants enduring and escaping from withering in drought condition [10], but Stocker [19], in addition, defined it as the ratio of yield in drought condition to that in optimal one. In this paper the term drought resistance will be used in the former meaning.

Another approach to the problem of drought resistance has been conducted by measuring osmotic values of cells or tissues. Walter [30] defined it as the product of maximal osmotic value and ability for maintaining osmotic value. This expression, as Walter himself remarked, does not show exact mathematical relations but only indicates the tendency of drought resistance. Besides, high osmotic value is often the result of high drought resistance, i.e., high osmotic value is the proof of endurance to extreme water loss. For this reason high osmotic value can be replaced by low lethal water content which is more convenient for the quantitative estimation of drought resistance.

In the present paper the drought resistance of pine yearlings in dune regions will be investigated quantitatively from the viewpoint of water economy and some general considerations by mathematical analysis of drought resistance in pine yearlings will be left to another paper [26].

The investigation was conducted at the Botanical Institute, Faculty of Science, University of Tokyo.

## I. THE HABITAT OF PINE YEARLINGS IN SHŌNAN AND ARAI DUNE REGIONS

The investigations consist of field researches and laboratory experiments. The former were chiefly conducted at Shōnan dune, but a part of them was also carried out at Arai dune.

The details of the habitat in Shōnan dune region were already mentioned in another paper [7], so only brief outlines will be sketched. This region is situated at the north coast of Sagami Bay, about 50 km. south-west of Tokyo, extending for 5 km. from Chigasaki City to Enoshima Island with the width of 0.7 km. in average. The inner part of western and eastern part of the dune was inhabited and most of the vegetation had been destroyed. Field researches were done partly at the front dune at the east end of the region (Kugenuma region), and partly in the inner part of front dune at the middle part of the region where vegetations were kept intact with rear dune developed in its western half (Chigasaki region). The latter region consisted of two parts, i.e., a) *Pinus Thunbergii*



stands at the eastern half and b) *Pinus Thunbergii* barrens at the western half. In all parts of the region front dune is well developed.

No higher plants could be found between the beach and south foot of the front dune. At and around the top of front dune several dune plants were dotted here and there with the afforestation of pine seedlings protected by sand heaping fences. The dominant species was *Carex Kobomugi* accompanied by *Phellopterus littoralis*, *Zoysia macrostachya*, *Lathyrus maritimus*, *Calystegia Soldanella*, *Lactuca repens*, *Ischaemum antheophoroides* var. *eriostachyum*, *Digitaria ciliaris*, *Fimbristylis sericea*, *Chenopodium acuminatum* var. *japonicum* and *Diodia teres* var. *setifer*, and the plant communities may be named *Carex Kobomugi* Comm. and *Pinus-Carex Kobomugi* Comm. (newly afforested parts). The last species, *Diodia*, was introduced from North America comparatively recently and spread rapidly in Shōnan dune region. This part belonged to Mobilideserta, the vegetation repeating exposure and burying throughout the year and no pine yearlings could be found even in germinating season.

Northern foot of front dune is adjacent to *Pinus Thunbergii* stands at the east part of Chigasaki region. The stands were covered by main tree-crops of about 20 year old with a height of 5 m. in average. Typical dune plants such as *Carex Kobomugi*, *Fimbristylis sericea*, *Zoysia macrostachya* and *Lactuca repens* disappeared among the undergrowth. At drier sites the dominants of undergrowth were *Imperata cylindrica* var. *Koenigii*, *Lespedeza sericea* and *Indigofera pseudotinctoria* and at the innermost part *Polygala japonica*, *Potentilla chinensis*, *Cyperus microiria* and *Ophiopogon japonicus* were added, which are common plants found in fields and hills of central Japan (*Pinus-Imperata-Lespedeza* Comm.). At wetter sites the undergrowth was characterized by the appearance of *Arundinella hirta* with the dominance of *Imperata* (*Pinus-Imperata-Arundinella* Comm.). Lastly, at marshy sites such hygrophytes as *Phragmites communis*, *Carex pumila* and *Salix* sp. appeared in addition to *Arundinella*, and *Imperata* almost disappeared (*Pinus-Arundinella-Phragmites* Comm.). Pine seedlings with the height of 0.2–0.5 m. sporadically grew in the former two communities especially along the road side. Also pine yearlings could be found everywhere from spring to early summer, whose fate will be mentioned afterwards. The vegetation of pine barrens neighbouring the pine stands presented different physiognomy. Tree-crops with the height of 1.5–2.5 m. were only found at the north slope of front dune and no seedlings grew under them. The dominants of undergrowth were *Zoysia* and *Imperata* accompanied by *Carex Kobomugi*, *Chenopodium acuminatum* var. *japonicum*, *Calystegia*, *Phellopterus*, *Ischaemum*, *Lathyrus maritimus*, *Fimbristylis*, *Digitaria*, *Bulbostylis* and *Diodia* (*Pinus-Zoysia-Imperata* Comm.). The lowland between front and rear dune was covered by dwarf pines with a height of 0.5–1.5 m. The most abundant herb was *Imperata* and the undergrowth was different from that of pine stands in the abundance of such dune plants as *Phellopterus*, *Fimbristylis* and *Zoysia*, and in the disappearance of *Indigofera*, one of the dominants of *Pinus-Imperata* Comm. At rear dune dwarf pines practically disappeared and a typical dune plant of *Carex Kobomugi* reappeared instead. Accompanying species were *Phellopterus*, *Imperata*, *Fimbristylis*, *Diodia*, *Lespedeza* and *Lactuca repens*, thus reviving the physiognomy of front dune except for the vast

crowd of *Imperata* (*Imperata-Carex Kobomugi* Comm.). The north foot of rear dune is adjacent to farmland. In Chigasaki region chief experiments were conducted in *Pinus-Imperata-Lespedeza* Comm., especially Plot 9 in the middle part of the stands.

Now, let us turn to another dune region situated near Arai Town, Shizuoka Prefecture. Arai dune region is situated at the north coast of Enshûnada, about 250 km. west-west-south of Tokyo, neighbouring Lake Hamana, an inlet famous for its scenic beauty. A part of the dune belonged to the University Forest of Tokyo University. The University Forest was separated into two stands, Fukiyoseshita and Matsuyama stands. The larger one was Fukiyoseshita stand, extending for 2.5 km from Hamana to Benten with a width of 100 m. in average. At 250 m. from the beach came the top of front dune whose north foot is adjacent to narrow lowland interrupted by rear dune at the northern border. The distance of the tops of both dunes was only 100 m. or thereabout. The northern foot of rear dune is adjacent to farmland across old pine stands of more than 100 year old. No plants could be found till 60-80 m. from the beach, and the area between this point and the top of front dune was scattered with dune plants similar to Shônán region. Near the top of front dune pine stands were afforested in a narrow strip by the reforestation section of Prefecture. The area



Fig. 1. N-S cross section of the Arai University Forest, Plot 14. 1. *C. Kobomugi* Comm., 2. Afforested parts by prefectural authorities, 3. Poor stands, 4. Excellent stands, 5. *Imperata* marsh, 6. Good stands, 7. Grass land, 8. Old pine trees.  
\* Chlorine content (mg. Cl/100 g. dry soil) for the corresponding parts in Plot 8.

between the tops of both dunes belonged to the University Forest. The planting was conducted during the interval of 1929-1941, one or two plots at a year from

TABLE 1  
The condition of pine stands afforested in 1936 (Arai dune)

Habitat	Plot 14 Lowland	Plot 14 Lowland	Plot 14 Lowland	Plot 15 Front dune slope	Plot 15 Front dune slope	Plot 15 Front dune slope
Height (m.)	5.5	6.0	5.3	1.3	1.3	1.0
Basal diameter (cm.)	5.8	6.5	8.3	3.7	3.5	3.3
Stem weight (kg.)	13.32	17.23	23.23	0.59	0.60	0.28
Branch weight (kg.)	3.81	3.04	16.83	0.58	0.53	0.36
Leaf weight (kg.)	3.75	4.47	13.41	0.74	0.93	0.41
Root weight (kg.)	7.29	10.82	27.96	1.44	1.55	0.91
Condition	standard	standard	dominant	inferior	inferior	inferior

the west to east parts. The number of plots was 21 and they were numbered from the west. The lowland between both dunes was marshy at the middle part of the stands. The growth of stands was extremely irregular, consisting of excellent and good stands with dark-green leaves and poor ones with yellow-green leaves. Most excellent stands were found in the marshy lowland and good stands were seen on the south slope of front dune. In other parts of lowland and slope the growth was generally poor. Abundant species under poor pine stands of dune slopes were *Carex Kobomugi*, *Ischaemum*, *Fimbristylis*, *Phellopterus* and *Calystegia* with absence of *Imperata*. In most marshy places afforested pines had been already killed and the soil was covered by the vast crowd of *Imperata* and *Carex arenicola*. In the excellent stands of lowland the undergrowth was utterly lacking or a few number of *Imperata*, *Lespedeza*, *Ischaemum* and *Carex pumila* were scattered here and there. In other good stands of lowland and at dune foot, *Imperata*, *Lespedeza*, *Ischaemum* and *Carex pumila* were abundant. In this region the existence of *Imperata* was always accompanied with good pine growth. The north slope of rear dune was already invaded by some inland herbs. The undergrowth of this region was characterised at first by the absence of *Diodia* and next by no clear difference between the dune vegetation and the undergrowth of pine stands, most of the typical dune plants penetrating deeply into the pine stands. As in the case of Shōnan dune region we could hardly find any pine seedlings germinated from fallen seeds.

## II. EXPECTED LIMITING FACTORS FOR THE GROWTH OF PINE YEARLINGS IN DUNE REGIONS

One of the environmental factors peculiar to coastal dune regions must be chlorine factor derived from sea water. So it may be tentatively assumed that the fate of pine yearlings of these regions has something to do with the chlorine content of the soil. Chlorine may be contained in sea breeze which adheres to plants and soil, entering into soil with rain water. In fact, pine leaves of outermost places have salty taste. Relative values of chlorine in sea breeze were estimated by exposing a definite size of gauze in vertical position at various parts of Shōnan dune region [7]. The result was that at 2 m. above soil surface the values rapidly decreased till 100 m. from the beach and from this point inwards the values were nearly constant. So, chlorine content in sea breeze at pine stands behind the front dune proved to be small as was expected. Also the filtration by tree-crops of chlorine in sea breeze was ascertained by measuring the chlorine amount attached to pine leaves and the relative chlorine in sea breeze at wind and lee side of a small pine stand near the front dune of Kugenuma region. The variation of chlorine content in soil with distance from beach conformed well with that in sea breeze, i.e., in a fine day during summer it was 9 mg. for 100 g. oven dry soil in places adjacent to the beach with no plants, while the values were small and rather uniform, i.e., about 1 mg. in the same unit from the south slope of front dune (*Carex Kobomugi* Comm.) to inner pine stands. Chlorine content increased after rainfall owing perhaps to the washing down of the chlorine attached to aerial parts and soil surface. In the soil of



pine barrens in Chigasaki region the content was also uniform and somewhat larger, i.e., 1.5–2.0 mg. [7]. The content and distribution of chlorine in the soil at the middle part of Fukiyoseshita stand of Arai region again gave same results (Fig. 1).

The concentration of chlorine in soil water will have direct influence upon the growth of pine yearlings, especially that of root, as most of the chlorine in the soil is dissolved in soil water. Even if the chlorine content for dry soil was the same, its concentration varies to a great extent by the water content of the soil, e.g., when chlorine content is 1 mg. for 100 g. dry weight, its concentrations at water content of 1, 2 (wilting percentage), 4, 6 (field water capacity), 10 and 30% (maximum water capacity) are, respectively, 0.100, 0.050, 0.025, 0.017, 0.010 and 0.003%. Vertical distribution on weight basis of chlorine content showed no significant variations, whereas the concentration of chlorine was always highest in the surface soil, which was due to the vertical variation of water content of soil.

So far the chlorine condition of the habitat of pine yearlings was sketched. The cardinal point is that how this chlorine condition has influences upon the germination and later growth. In a previous paper of the author [22] no inhibiting effect could be observed both for germination and growth up to a chlorine content of 10 mg. for 100 g. oven dry weight. By this chlorine content its concentration is calculated to be 0.10–0.25% when soil water content fluctuates between 4–10%, i.e., the water content to which the roots of the yearlings are exposed most of the time in their habitat. Comparing this chlorine content and those of their habitat, chlorine factor *per se* can not be the limiting factor for the growth of pine yearlings in dune regions on account of the high salt tolerance of pine yearlings.

In the soil of dune regions the shortage in mineral substances, especially in nitrogen, is expected which may have some relations to the growth of pine yearlings in their habitat. Secondly, the amount of organic substances alters the constants concerning water relation and the content of nitrogen may be admitted as an index of the amount of organic substances in the soil. For this two reasons the local and vertical distribution of nitrogen and nitrogenous compounds was studied in both dune regions. Total-N was determined by semimicro-Kjeldahl's method and albuminoid-N by colorimetric determination of ammonia in steam distillate of alkaline  $\text{KMnO}_4$  solution containing the sample [33]. Next, ammonium-N was determined also colorimetrically with steam distillate of soil extract. 10% KCl extract yielded tenfold ammonium-N of water extract owing to ammonium-N adsorbed on the surface of soil colloid particles. The samples were extracted by 10% KCl before distillation. Lastly, nitrite-N was determined by water extract of the soil with colorimetric method using Gries-Romijn reagent as colour former [11].

Vertical distribution of nitrogenous substances was estimated chiefly in Arai dune, but a few estimations were also conducted in Shōnan dune. In Arai dune, samples were taken from west part (Plot 5) with extremely heterogenous growth, and from middle part (Plots 14, 15) with good growth as a whole. As a check a place adjacent to the beach was added. In pine stands of Chigasaki dune samples were gathered from a drier site of *Pinus-Zoysia* Comm. (Plot 4) and *Pinus-*



*Imperata-Lespedeza* Comm. (Plot 9). The tendency of distribution was similar in total- and albuminoid-N, i.e., in good stands the content showed maximum value at the soil surface, then it was kept constant or decreased slowly till to the depth of 90 cm. Excellent stands (1 and 6 in Fig. 2) have a higher amount of nitrogen even at a deeper point. In poor stands the content was small even at soil surface in most cases, the values being uniform to deeper points. Minimum content was determined in places near to the beach (Fig. 2). The content of ammonium- and nitrite-N also decreased at a deeper position but their values were, respectively, one and three orders smaller than that of total nitrogen (Table 2).

In various soils of Japan, from archean to quaternary soil, the content of total-N was between 1.34 and 4.91 mg./g. dry weight, the average being 2.28 mg. [15]. Also by the courtesy of Prof. Matsumoto of our University, the total-N content of the upper layer of Kantô loam soil of Fuchû City, Tokyo, was usually 2-3 mg. Compared with these values those of dune regions are one order smaller even in the surface layer and the shortage of nitrogen was prominent. But nitrogen factor may not be the

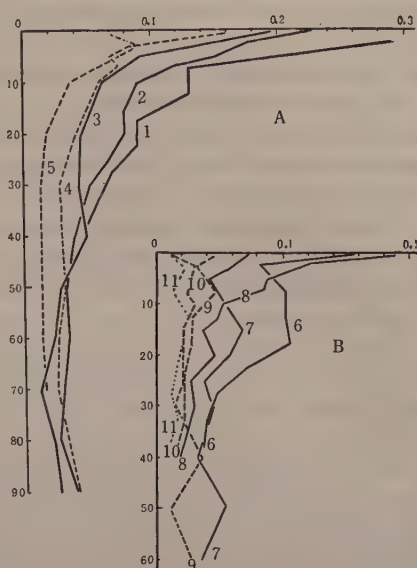


Fig. 2. Vertical distribution of total (A) and albuminoid (B) nitrogen in Arai and Chigasaki dune regions. Solid line, good stand; Broken line, poor stand; Dotted line, beach side. Arai—1, 3, 4, 5, 6, 7, 9, 10, 11; Chigasaki—2, 8. Ord. depth in cm.; Absc. mg./g. dry soil.

TABLE 2  
Vertical distribution of Total-,  $\text{NH}_3$ - and  $\text{NO}_2$ -N of the good stand (Plot 5) in Arai dune

Depth cm.	Total-N	$\text{NH}_3$ -N	$\text{NO}_2$ -N
	mg./g. dry soil		γ/g. dry soil
0-0.5	0.75	0.012	0.078
0.5-5	0.29	0.007	0.036
5-10	0.13	0.006	0.019
10-15	0.13	0.007	0.021
15-20	0.09	0.005	0.019
20-25	0.09	0.007	0.019
25-30	0.07	0.004	0.015
30-35	0.06	0.005	0.028
35-40	0.05	0.006	0.018

limiting factor for the growth of pine yearlings, for they ordinarily grow in good condition in containers as well as in their habitat without the supply of nitrogenous compounds provided that the water relation of the soil was kept in good condition. Also such a small amount of organic substances as was inferred from total-N content can scarcely influence the water content of the soil which was proved to be true in the following investigations.

In habitats inside the pine stands the influence of light condition may play some role on the growth of the yearlings. Relative light intensity inside the stands, however, was 50% in average. So the qualification of light factor as a limiting factor may be denied.

After examining in vain the qualification of some environmental factors for the limiting factor of the growth of pine yearlings, let us proceed to examine water factor, the most probable limiting factor for the growth of pine yearlings.

### III. WATER FACTOR AS THE LIMITING FACTOR FOR THE GROWTH OF PINE YEARLINGS IN DUNE REGIONS

#### 1. *Soil constants concerning water relations*

As green plants absorb water almost exclusively from soil, soil moisture is the most direct environmental factor for the growth of green plants. Soil moisture is derived mainly from rain water, but a part of rain water is lost by canopy interception, i.e., the evaporation of water attached to the aerial part of plants during rainfall. Out of rain water entering into the root layer of plants, a part is again lost by surface run-off, soil surface evaporation and percolation, thus water added to root layer during and soon after rainfall becomes, precipitation amount minus those measures above mentioned.

The annual precipitation of Kanagawa Prefecture (Yokohama) and Shizuoka Prefecture (Hamamatsu) are, respectively, 1657 and 1880 mm. with monthly peaks in June and September, being the Pacific pattern of summer rain. The mean relative humidity attains to its maximum also in summer (Table 3). In spite of the

TABLE 3

Monthly precipitation and mean relative humidity of Yokohama (Kanagawa Pref.) and Hamamatsu (Shizuoka Pref.) based on the measurements between 1921 and 1950. (From *Chronical Table of Science*, 1959)

Monthly precipitation (mm.)

Month	1	2	3	4	5	6	7	8	9	10	11	12	Total
Yokohama	45	81	104	145	146	186	137	164	253	230	101	64	1657
Hamamatsu	52	75	139	162	177	246	195	183	273	198	111	68	1880

Monthly mean relative humidity (%)

Month	1	2	3	4	5	6	7	8	9	10	11	12
Yokohama	64	65	68	73	77	82	83	82	82	80	74	68
Hamamatsu	62	61	63	71	75	81	84	82	81	75	70	66

plentiful monthly precipitation in summer the dune regions are often threatened by drought in this season. What is the reason for this phenomenon? The answer is that the monthly amount of precipitation is large in one rainfall, while the interval between two succeeding rainfalls is long enough to cause drought condition. The dry intervals ensue not uncommonly for two weeks. The concrete rainfall will be mentioned in detail afterwards. In dune regions the amount of surface run-off can be omitted. Next, canopy interception was estimated by main tree-crops in Chigasaki region [14] [23]. Canopy interception is the sum of water storage capacity (maximum amount of rain water attached to the aerial plant parts) and evaporation during the rainfall. The water storage capacity of an excellent tree-crop of this region was only 1.25 mm. for crown projected area and those for inferior tree-crops are much smaller. By the precipitation rate of 3 mm. an hour, accumulative interception rate was computed to be 26% for initial 2 hours and was reduced to 7% after 24 hours of rainfall. Low percentage in long intervals of rainfall is due to the limited amount of storage capacity and also to the small amount of evaporation from aerial parts during rainfall. The loss by canopy interception, therefore, plays no significant role in long intervals of rainfall. Soil surface evaporation during rainfall is negligible likewise the evaporation from plant parts. Lastly, we are not driven by necessity to measure the amount of percolation, for the water content of root layer after excess water has moved downwards by percolation, i.e., field water capacity, is always constant, this content being 27 mm. when 30 cm. depth of surface soil is assigned to root layer and field water capacity is 7% on oven dry basis. Suppose again the rainfall with the rate of 3 mm. an hour. The relation between precipitation amount and interception was already calculated for a pine stand of most closed condition [23]. Then the distribution of precipitation was as shown in Table 4, assuming that the root layer had 13.5 mm. of water amount

TABLE 4

Calculated distribution of precipitation amount under the precipitation rate 3 mm. for an hour in Chigasaki dune. As the root layer, surface soil of 30 cm. depth was assigned, whose water content at FC corresponds to 27 mm.

It was assumed that the root layer had 13.5 mm. water at the beginning of rainfall

Duration of rainfall (hrs.)	Precipitation amount (mm.)	Interception		Soil surface evaporation		Water added to root layer		Percolation	
		mm.	%	mm.	%	mm.	%	mm.	%
2	6	1.58	26.4	0.02	0.3	4.40	73.4	—	—
3	9	1.75	19.4	0.03	0.3	7.22	80.3	—	—
5	15	2.08	13.9	0.06	0.4	12.86	85.6	—	—
7	21	2.41	11.5	0.08	0.4	13.50	64.3	5.01	23.8
10	30	2.90	9.7	0.11	0.4	13.50	45.0	13.49	45.0
15	45	3.73	8.3	0.13	0.3	13.50	30.0	27.64	60.7
20	60	4.55	7.6	0.22	0.4	13.50	22.5	41.73	68.6
24	72	5.21	7.3	0.26	0.5	13.50	18.7	53.03	72.2



before rainfall, which was usually the case in summer, and that field water capacity was 7% on dry weight basis. From the table it will be noticed that the rate of soil surface evaporation was constant and small over the whole range of duration and that the rate of interception steadily decreased as the duration prolonged, while water added to root layer increased at comparatively light rain not enough to cover the field water capacity, but again decreased in heavy rain with simultaneous increase in percolation. From these it can be concluded that a considerable part of rainfall was lost by canopy interception in light rains and by percolation in heavy ones, only a part of the precipitation can being caught by root layer.

Only available water can be absorbed and utilized by higher plants. Of utmost importance as the borderline between non-available and available water is the wilting coefficient or wilting percentage originally established by Briggs and Shantz [2] in 1912. The author [25] measured suction pressure in various water content of dune soils by Hansen's method [3] with some modifications. With the decrease of soil water content suction pressure at first slowly and then abruptly increases, the water content with suction pressure of 15 Atm. or pF 4.2 being defined as critical moisture [9] which coincides well with wilting percentage. Critical moisture or wilting percentage, thus determined, was 2.0 and 1.0%, respectively for Shōnan and Arai dune soil. Next, the maximum water capacity of both dune soils was 30 and 28% as measured by the conventional method adopted in Japan. The water content of root layer in these regions never reached up to this constant and was always far smaller, the significance of this constant, therefore, was small as in the case of hygroscopic coefficient. In spite of the temporal increase of water content in surface soil during and just after the rainfall, the gravitational water soon drains away and only a part of rain water is left in the soil. This amount is a constant in one soil under a given condition and was named by Veihmeyer and Hendrickson [28] as field water capacity (FC). Available water capacity (AWC) may be defined as the water amount between FC and wilting percentage, which is a sort of constant for given soils and the term, 'available water' should be reserved for the difference between arbitrary water content and WP. The author estimated the FC of both dune regions by measuring the vertical distribution of soil moisture, which was 6-7% and 4-5%, respectively for Shōnan and Arai dune regions.

So far, the soil constants of water relations have been expressed on oven dry basis for convenience sake. For ecological studies, however, the volume basis is more preferable, as the available water for unit volume of soil has direct connexions with water economy of plant communities. To convert soil water content from oven dry basis to volume basis the dry weight of unit volume of soil, i.e., volume weight, is necessary for calculation. Thus, the dry weight of a definite volume of soil with original structure was weighed to determine the volume weight. The results are tabulated in Table 5. The values were about 1.5 g./cm<sup>3</sup>., the variation by habitat and soil depth being insignificant. Then, if we put water content (% on oven dry basis) and volume weight as  $W_a$  and  $\rho$ , the percentage of water content on volume basis ( $W_v$ ) will be,  $W_v = W_a \rho$ . In water content below FC the movement of water in the soil is so sluggish that

TABLE 5

Volume weight (g./cm<sup>3</sup>) of Chigasaki and Arai dune soils at various depth from soil surface. Each value is the average of 4 or 5 measurements

Depth under soil surface (cm.)	Chigasaki, Plot 9, good stands	Chigasaki, Plot 4, poor stands	Arai, Plot 15, rear dune slope, good stands
0	1.53	1.60	—
5	1.47	1.56	1.47
10	1.47	1.48	1.49
20	1.44	1.53	1.46
30	1.45	1.48	1.57
40	1.42	1.46	1.56
50	1.40	1.45	—

the roots can practically gather water only from the adjacent part of the soil, in other words, it may not be too much to say that the plants can only absorb available water in their root layer. For this reason we are driven by necessity to estimate the total available water in root layer, then it is desirable to express the amount of available water in terms of mm. in order to establish the connexion with the amount of precipitation. For the conversion of water amount from  $W_v$  to  $W_{mm.}$  (water amount expressed in mm.), the depth of root layer is put as  $h$  cm. and the structure of soil is assumed to be vertically uniform, then the water amount in a square pillar of soil ( $1 \times 1 \times h$  cm.) becomes  $W_v h / 100$  g. or  $W_v h / 10$  mm. Putting the value of  $W_v$  in the previous equation, water amount in mm. of the root layer will be  $W_{mm.} = W_a \rho h / 10$ . From this formula the available water in root layer can be calculated from water content on oven dry basis and volume weight. If soil structure varied with the depth  $W_{mm.}$  can be estimated as the summation of  $W_{mm.}$  in every layer. Also,  $W_{mm.}$  of root layer can be obtained by integration when the water content of soil layer varies vertically.

As the soil structure was vertically and horizontally almost uniform in the soils of both dune regions, the available water capacity (AWC) of root layer can easily be calculated from above formula. In the case of Shônan dune FC and WP can be put as 6 and 2% on oven dry basis, AWC being  $6 - 2 = 4\%$ . As will be mentioned afterwards most of the roots of main tree-crops and undergrowth lay between soil surface and 30 cm. depth, so 30 cm. was assigned to root layer ( $h$ ). Putting  $W_a = 6 - 2 = 4$ ,  $\rho = 1.5$  and  $h = 30$  in the formula, the available water capacity of root layer was calculated to be 18 mm.

It may be of interest to compare the soil constants concerning water relation of both dunes with those of other soils. Fortunately the author has in hand the data of those in Kantô loam soil of Koganei, Tokyo, a soil derived from volcanic ashes of the diluvial epoch. This soil consists of black surface soil down to the depth of 40 or 50 cm. with red-brown subsoil beneath. The vertical distribution of soil water content in this soil was somewhat different from that in sand dune soils, water content steadily increasing as the depth and the constancy of water content over whole range of depth could never be observed owing to the gradual variation of soil structure with the depth. In black surface soil, however,

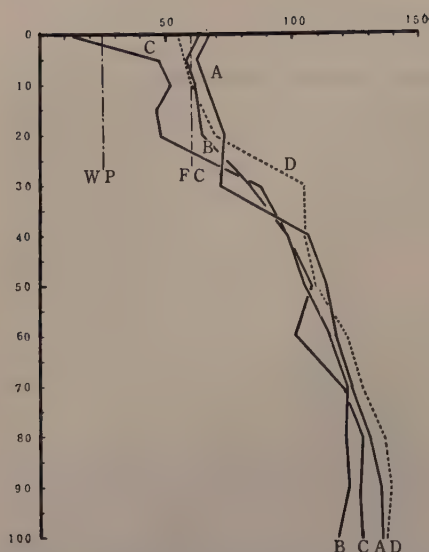


Fig. 3. Vertical distribution of soil water in Kantô loam soil of Koganei, Tokyo. A. 16h. on July 26, 1958 during 25.3 mm. rainfall B. 15h. on July 27, 1958, the next day after rainfall C. On June 4, 1958, extremely dry condition D. Winter condition. Ord. depth in cm.; Absc. water content, % on oven dry basis.

the water content was kept comparatively constant after rainfall, which was about 60% on oven dry basis and was 50% even in extremely dry seasons. From these the FC of surface soil was estimated to be 60%. The values of WP, MC and volume weight were, respectively, 25%, 100% and  $0.9 \text{ g./cm}^3$  by author's measurements. Comparing the constants of dune soils with those of Kantô loam soil, WP, FC and MC are surprisingly small in the former for oven dry, as well as for volume basis, the AWC of the former being only a ninth part of the latter in oven dry basis, and the difference is a little alleviated by the difference of volume weight in volume basis (1:5). As the AWC of soils down to a definite depth expressed in mm., say 30 cm., is proportional to water content on volume basis, dune soil has only a fifth part of available water in root layer compared with Kantô loam soil. In Table 6 some of the data in different soils were gathered. Only Russel's data were complete, but it must be born in mind that he measured water equivalent instead of FC, no conformance of the

TABLE 6  
Soil water constants in various soils expressed as oven dry basis. WP, wilting percentage; FC, field water capacity; MC, maximum water capacity; AWC, available water capacity; \* water equivalent

Soil	Oven dry basis, %				% of MC			Author
	WP	FC	MC	AWC	WP	FC	AWC	
1. Chigasaki soil	2	6	30	4	6.7	20.0	13.0	Tazaki
2. Arai soil	1	4	28	3	3.6	14.3	10.8	Tazaki
3. Sand soil	1.4	—	28.9	—	—	—	—	Livingston & Kôketsu
4. Clay soil	10.4	—	56.8	—	—	—	—	"
5. Humus soil	70.7	—	140.5	—	—	—	—	"
6. Sand soil	3.7	7.6*	44.5	3.9	8.3	17.1	8.8	Russel
7. Sandy loam soil	7.2	15.6*	58.0	8.4	12.4	27.0	14.6	"
8. Clay soil	20.6	30.4*	87.0	9.8	23.6	34.9	11.3	"
9. Loam soil	9.0	17.0	—	8.0	—	—	—	Wedleigh et al.
10. Clay soil	17.0	54.0	—	37.0	—	—	—	Richards & Weaver



two being observed in sandy soils as has been verified by various investigators [8]. From the table it will be observed that the AWC of our dune soils belongs to one of the smallest ones ever studied.

It has been a custom in soil water study, especially in applied field, to express the water content of soil as the percentage of maximum water capacity in

the control for water content of pots etc. The inadequacy of this custom for the study of water economy of plants will be clear from Fig. 4. Suppose the water content, 20% of maximum water capacity. In Kantô loam soil this value is below WP, i.e., plants can hardly absorb water from soil at this content, while in dune soils this value, ironically enough, corresponds to FC, i.e., the upper limit of water content concerning availability. For this reason soil water content should be expressed in relation

to its availability for plants, i.e., to the amount of available water on volume basis or mm. In the following sections, however, water content will be often expressed on oven dry basis as the determination of water content is always done in this unit and the relation of water content to WP and FC was made clear in this section.

## 2. The influences on soil water content of soil surface evaporation and transpiration of main tree-crops and undergrowth

After enough precipitation surface soil retains water content corresponding to FC. This water content is depleted both by soil surface evaporation and transpiration of main tree-crops and undergrowth so long as rainless days continue. For this reason it is necessary to make clear the role of both factors in the variation of the water content of surface soil in order to substantiate the vertical distribution of soil water as will be mentioned in the following section.

At first, the variation of soil surface evaporation with the drying of soil was experimentally determined by a preliminary experiment. The soil of Shônan dune was packed into specimen bottles with inner diameter of 6.3 cm. as near as possible to its original structure. Initial water content was about FC and evaporation was measured in comparatively mild condition of April by weighing bottles at noon every day. The amount of five parallel experiments were averaged, the amount of evaporation from water surface was measured as the check. The depth of grey coloured part under soil surface was measured as the depth of dry layer, because the discolouration occurred in this soil at the water content

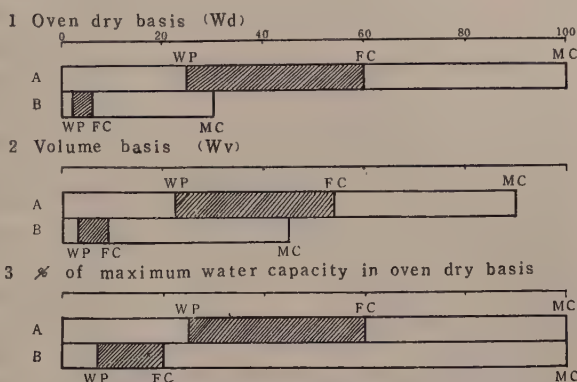


Fig. 4. Comparison of soil water constants of Chigasaki dune (B) with Kantô loam soil of Koganei, Tokyo (A). WP, wilting percentage; FC, field water capacity; MC, maximum water capacity; AWC, available water capacity. Hatched parts in the figure show AWC.

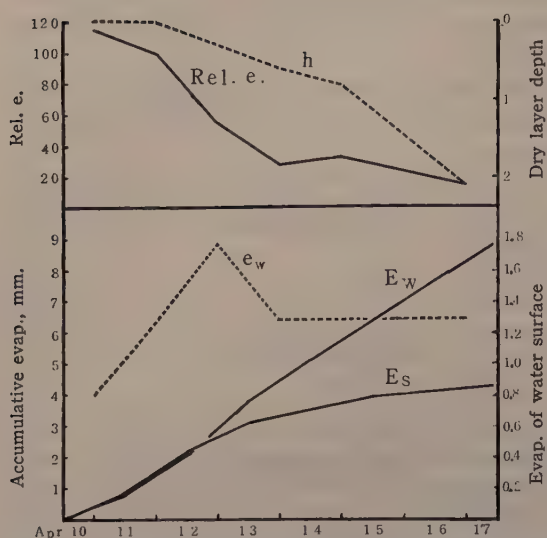


Fig. 5. Soil surface evaporation in the drying process.  $E_s$ ,  $E_w$  are accumulative evaporation of soil and water surface;  $e_s$ ,  $e_w$  are evaporation of soil surface and water surface in mm. for a day;  $h$  is the depth of dry layer (cm.). Relative evaporation (Rel. e.) is  $e_s/e_w \times 100$ .

soils with their original structure were packed into a brass soil-sampler for volume weight determination (evaporating surface, 100 cm<sup>2</sup> and depth, 4 cm.) In one experiment the soil was sampled at the depth of 15 cm. with water content near to FC, in another experiment upper 1 cm. of the sampled soil was substituted for equal depth of dry soil, and in still other experiment upper 2 cm. was substituted. In some cases intact upper soil was packed. The samplers with soils were then buried in the soil at sunny sites, the evaporating surface coinciding with soil surface and the amount of evaporation was determined by weighing the container at different intervals, mostly 2 hours. As a check evaporation from water surface was also determined. The results were illustrated in Fig. 6 of the daily variation of evaporation measured every two hours from 9 to 17 hrs. The variation of water surface evaporation (a) took similar course with those of soil surface temperature and saturation deficit in the shade. Evaporation from natural soil surface (c) was much smaller than the former and varied only a little throughout the daytime and the result was quite the same in soil surface with 1 cm. of dry layer (d). Lastly the evaporation from wet soil surface (b) was comparatively large in the beginning but diminished considerably as the surface was dried and fell to the level near to that of natural soil surface in the afternoon. Total amount of evaporation from 9 to 17 hrs. in natural soil surface (0.88 mm.) was much smaller than that in water surface (4.43 mm.), and the dry layer near the soil surface was already a little less than 1 cm., though August 26 was the next day after previous rainfall (118 mm. on Aug. 24 and 2 mm. on Aug. 25). As this

of WP. As seen from Fig. 5 the soil surface evaporated as much as, or more than water surface when the soil was wet up to the surface. But the ratio of soil surface evaporation to water surface evaporation, i.e., relative evaporation, began to decrease as soon as dry layer appeared and fell to below 20% when the depth of dry layer was 2.1 cm. So the accumulative evaporation of both evaporating surface took a different course. In the habitat it was often observed that the dry layer appeared on the next day after rainfall, so the surface drying may play a large part in keeping the soil moisture. Let us turn to the experiments conducted directly in the habitat. The chief experiments were done at coastal pine stands of Kugenuma region during the summer of 1949. The

evaporation amount was similar to the evaporation from soil surface with 1 cm. of dry layer, the latter may be regarded as the evaporation of soil surface adjacent to the previous rainfall. That the amount of evaporation from wetted soil surface fell to the level of those with dry surface may show the variation of evaporation immediately after rainfall, i.e., the evaporation may diminish to a considerable extent within a day. Same sort of experiments were repeated during August and September of that year. Total amount of evaporation from 9 to 17 hrs. was illustrated in Fig. 7. In accordance to weather conditions evaporation from water surface fluctuated to a considerable extent,

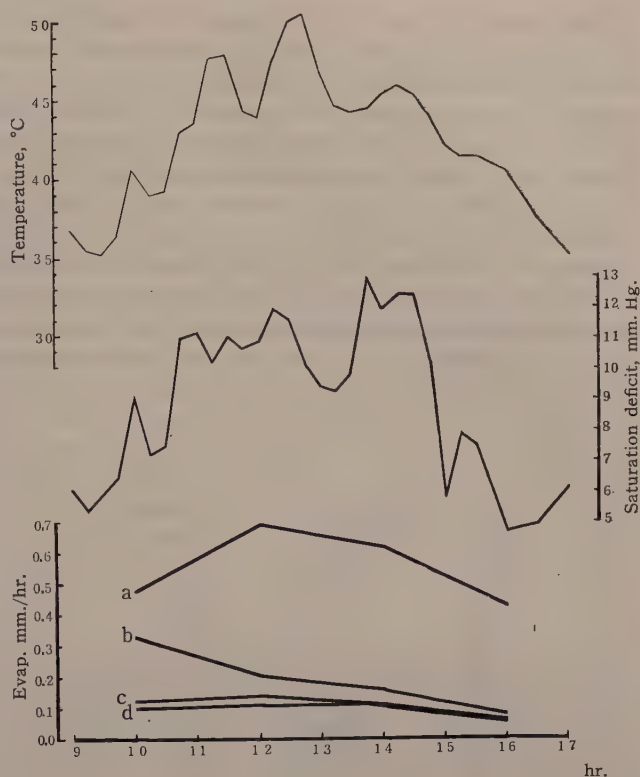


Fig. 6. Soil surface evaporation measured at pine stands of Kugenuma region. a. water surface, b. soil wetted to the surface, c. natural soil d. soil with 1 cm. of dry layer.

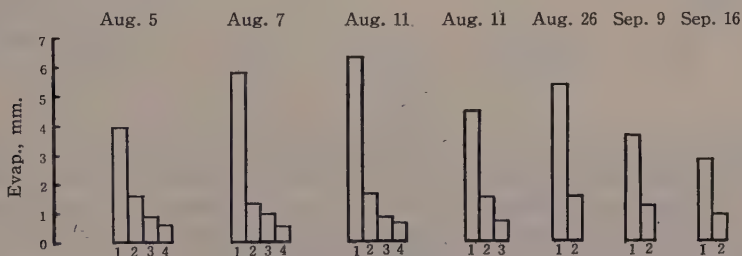


Fig. 7. Soil surface evaporation measured at pine stands of Kugenuma in the summer of 1949 from 9 to 17hrs. on fine days. 1. water surface, 2. soil wetted to the surface, 3. soil with 1 cm. of dry layer, 4. soil with 2 cm. of dry layer. At noon, soil surface temperature was 35.5-55.0°, saturation deficit in the shade was 7.10-14.59 mm. Hg.

while soil surface evaporation was nearly constant at least during August, thus

Soil wetted to the surface	1.5 mm. for 9-17 hrs.
Soil with 1 cm. dry layer	0.8       "
Soil with 2 cm. dry layer	0.6       "



As the second soil surface evaporation corresponded to natural one in comparatively moist condition, the third one may show the evaporation in drier condition of soil. On fine days in summer the depth of 5 cm. of surface soil was not uncommonly dried up below WP, whose evaporation, though could not be determined by our method, may be conjectured to be less than 0.6 mm. during 9 and 17 hr. Taking into account also the evaporation before 9 hr. and after 17 hr., it might be, nevertheless, an overestimation to put daily soil surface evaporation as 1 mm. a day.

Secondly the water content of surface soil is exhausted by the water absorption, therefore by the transpiration, of main tree-crops and undergrowth. For the purpose of making clear the water absorbing zone by roots of tree-crops and undergrowth the standing crop of roots in an area of 1m.<sup>2</sup> between main tree-crops was determined after stratifying roots into strata of 10 or 5 cm. (Fig. 8). With a few

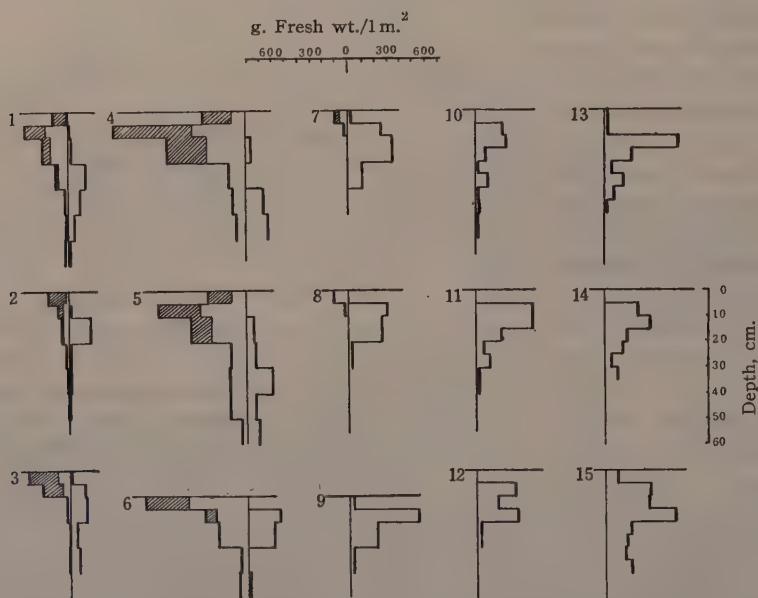


Fig. 8. Vertical distribution of underground parts of pine main tree-crops and undergrowth in Chigasaki and Arai regions. Pine roots are illustrated to the right and herbs (*Imperata*, 1-5; *Imperata*+*Arundinella*, 6; *Fimbristylis* 7; *Zoysia*, 8) to the left. Hatched parts are rhizomes. 1-3, Chigasaki *Pinus-Imperata* Comm.; 4-5, Chigasaki trenched plot in the same Comm. 6. Chigasaki *Pinus-Imperata-Arundinella* Comm.; 7-9, Chigasaki *Pinus-Zoysia* Comm.; 10-12, Arai poor stands, Plot 15; 13-15, Arai good stands, Plot 15.

exceptions most of the roots lay between the depth of 0-30 cm. The mass of the roots and rhizomes of *Imperata* in a trenched plot in *Pinus-Imperata* Comm. was outstanding. By trenched plot the author means a plot surrounded by dugout in order to cut out the influences to inside area of the roots of main tree-crops. By these means Toumey [27] firstly examined the role of the roots of main tree-crops upon the water consumption of forest soil in order to make clear the cause, light

versus moisture, of the fate of tree seedlings on forest floor. The author set up five trenched plots (3×3 m.) in *Pinus-Imperata* Comm. (Plot 9) of Chigasaki region in March, 1950. The depth of the dugout was 50 cm. and nearly all roots of the main tree-crops were cut off. The dugout was then buried to its original level to prevent the drying of inside area. Vertical distribution of soil water content was measured frequently from the spring to summer of that year. In most cases, especially in summer, water content inside the plot was somewhat higher than the outside for all range of depth, but the difference was insignificant. Whereas the standing crop of subterranean part of *Imperata* increased greatly, the suggestion being possible that improved water relation stimulated the growth of this plant, which again consumed soil water due to the increased transpiration of it and the result was that the water content was not so considerably increased as was expected.

In order to estimate the place of the transpiration amount of undergrowth, the standing crop of the shoots of undergrowth in 1 m.<sup>2</sup> area was estimated in *Pinus-Imperata* Comm. (Plot 6) of Chigasaki region during midsummer. The dominant of undergrowth was *Imperata* whose quantitative distribution was heterogenous, the fresh weight of leaves ranging from 20 to 130 g. for 1 m.<sup>2</sup> (Table 7). The standing crops of other frequent species were considerably small

TABLE 7

Standing crop in summer of undergrowth in *Pinus-Imperata* Comm. (Plot 6) at Chigasaki dune. Measured on July 27, 1959. The area of each quadrat was 1 m<sup>2</sup>

Species	Quadrat 1		Quadrat 2		Quadrat 3	
	F	C	F	C	F	C
<i>Imperata cylindrica</i>	18.5 g.	7.2 g.	42.8 g.	26.3 g.	128.0 g.	78.5 g.
<i>Carex arenicola</i>	7.6		—		17.3	
<i>Diodia teres</i>	1.0		1.0		3.9	
<i>Lespedeza serica</i>	—		9.0		7.2	
<i>Calystegia Soldanella</i>	4.3	1.2	—	—	—	—

compared with *Imperata*. Kadota [5] measured the transpiration amount of undergrowth in field condition for three species. The average amount in summer for *Imperata* was 5.75 g./g. fresh weight/day. Transpiration amount in mm. for one day calculated from above value was 0.11, 0.25 and 0.74 mm. for one day, respectively for Quadrat 1, 2 and 3. So the water consumption by transpiration of undergrowth may be unexpectedly small even in the densest community as Quadrat 3.

The influences of the transpiration of main tree-crops and young trees are more complex, as the roots of good tree-crops develop in two layers, the one near the soil surface (0–30 cm.) and the other at just above ground water level [7], water absorption, consequently, will be executed at two layers. In this case, therefore, it is impossible to determine the amount of water absorption from surface soil. But the situation was quite different in poor tree-crops with no

taproot, in which water absorption must depend solely upon the roots adjacent to soil surface. The amount of available water from soil surface down to the depth of 30 cm. was 18–22.5 mm. when FC was 6–7% on oven dry basis. Daily amount of transpiration was also estimated by Kadota [5] during the summer of 1950 in young stands of Kugenuma region afforested in 1946, which was 2–3 mm. in most cases. Then, total available water down to the depth of 30 cm. will be exhausted within a week or so in poor stands with no taproot provided that the soil moisture below the depth of 30 cm. is non-available. In midsummer the maximum length of rainless intervals was over 20 days, so the existence of young stands with the amount of transpiration above mentioned may be impossible if they had no taproot. Lastly what is the situation in surface soil of good pine stands, the native habitat of pine yearlings? In pine stands, as was mentioned earlier, available water is consumed firstly by soil surface evaporation and transpiration of undergrowth. As the daily amount of the former was in average below 1 mm. and that of the latter in medium stocked community was 0.25 mm. for *Imperata*, the total may be 1 mm. or thereabouts. The role of pine roots on the water consumption may be slight as can be conjectured from vertical distribution of pine roots (Fig. 8). Daily consumption of available water by 1 mm. or thereabouts for 20 days of rainless days can scarcely cover the storage of available water, 20 mm. down to 30 cm. from soil surface, and this consideration coincides well with the observed vertical distribution of soil water at the driest interval as will be mentioned in the following section.

### 3. Regional and seasonal fluctuation of the vertical distribution of soil water

As the direct environmental factor for water absorption of green plants vertical distribution as well as regional fluctuation, of soil water must be thoroughly investigated over the growing season. The measurement of soil water content was conducted for two years, from February, 1949 to January, 1951 at Shōnan dune region. For taking samples holes were dug to the depth of 70–100 cm, and in some cases to ground water level, from the interior part of which samples were taken and immediately packed into weighing bottles, the water content being measured in ordinary manner.

A variety of physiognomy developed before our eye in dune region of Chigasaki, the object of the author's research. When we turned our steps from the strand of the Pacific Ocean with surging billows to the top of front dune scattered with a few sand binding species, we were able to find close to our feet yellow green leaves of pine seedlings in newly afforested parts surrounded by sand defending fences. Across the coastal highway adjacent to the newly afforested parts the lowland between two dunes was partly covered by older pine stands and partly by pine barrens with dwarf pine and dune plants, behind these pine barrens lying the rear dune again with a similar aspect as front dune. Loitering in these regions it might be reasonable to think that the water condition of soil in various parts of this region was different. Twice in the spring of 1949, the water content of soil was determined at the depth of 5 cm. in various parts of pine stands in Chigasaki region (Table 8). From the table it will be observed that the water content was comparatively uniform, mostly between 6 and 8% on



TABLE 8

Soil water content at the depth of 5 cm. in various communities of pine stands in Chigasaki region, measured on Apr. 15, 1949, two days after rainfall

No. of Plots	Distance from beach (m.)	Main tree-crop		Water content (%)	Community name
		Height (m.)	Diameter breast high (cm.)		
1	60	—	—	5.55	<i>Carex Kobomugi</i> Comm.
2	80	—	—	7.43	<i>Pinus-C. Kobomugi</i> Comm.
3	140	1-2	—	5.72	<i>Pinus-Zoysia</i> Comm.
4	160	3-4	4.8-5.3	6.22	<i>Pinus-Zoysia</i> Comm.
5	180	1-2	—	5.85	<i>Pinus-Imperata-Lespedeza</i> Comm.
6	210	4-5	7.8	6.87	<i>Pinus-Imperata-Arundinella</i> Comm.
7	270	3-4.5	5.6	9.19	<i>Pinus-Imperata-Lespedeza</i> Comm.
8	340	4-6	8.9	12.38	<i>Pinus-Imperata-Arundinella</i> Comm.
9	370	4-5	6.2	6.88	<i>Pinus-Imperata-Lespedeza</i> Comm.
10	420	4	5.9	5.52	<i>Pinus-Imperata-Lespedeza</i> Comm.
11	480	5-6	14.1	8.97	<i>Pinus-Arundinella-Phragmites</i> Comm.
12	500	3-4	9.5	—	<i>Pinus-Imperata-Lespedeza</i> Comm.
13	520	5-6	13.1	—	<i>Pinus-Imperata-Arundinella</i> Comm.
14	520	4-5	8.1	5.10	<i>Pinus-Imperata-Lespedeza</i> Comm.
15	540	5-7	10.9	7.69	<i>Pinus-Imperata-Arundinella</i> Comm.
16	560	5-6	8.7	6.75	<i>Pinus-Imperata-Lespedeza</i> Comm.
17	600	3-4	7.1	6.54	<i>Pinus-Imperata-Lespedeza</i> Comm.

oven dry basis with few exceptions. In order to know more closely the regional difference the vertical distribution of water content was simultaneously measured in dry and wet pine stands (Fig. 9). The result was that also no significant difference was seen between the two stands. Sudden increase of water content at the lower layer in the wettest site was due to the approach to ground water level. Occasionally the soil surface of pine stands was covered by a thin layer of the decomposed product of organic substances (Fig. 2). The high amount of water content at soil surface on the next day after rainfall tells this fact. From the figure it will be observed that the vertical distribution of water content was uniform at every depth shortly after rainfall, while in fine days water content was remarkably diminished in the surface layer. Rapid decrease of water content in surface layer may be expected from prompt discolouration of soil surface after rainfall as was mentioned in the previous section, but in addition, vertical distribution of soil water was measured twice in a day, in early morning and in the afternoon in order to substantiate the velocity of drying (Fig. 10). On the following day after rainfall the water content of surface layer was somewhat larger than at other depth as was mentioned previously, whereas in the afternoon it was already decreased to a large extent, showing the drying, at least, of the surface layer was considerably rapid, but during rainless days no difference was practically

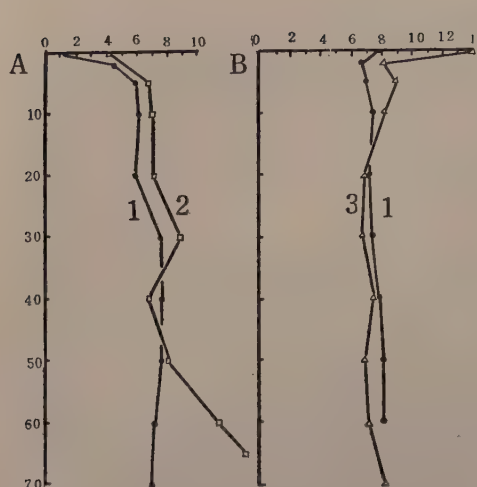


Fig. 9 Vertical distribution of soil water (Absc., %) in drier and wetter sites at Chigasaki region. A. May 12, 1949, a fine day, B. May 21, 1949, on the next day after rainfall. 1. Drier stands, 2. Wettest stands, 3. Wetter stands. Ord. depth in cm.

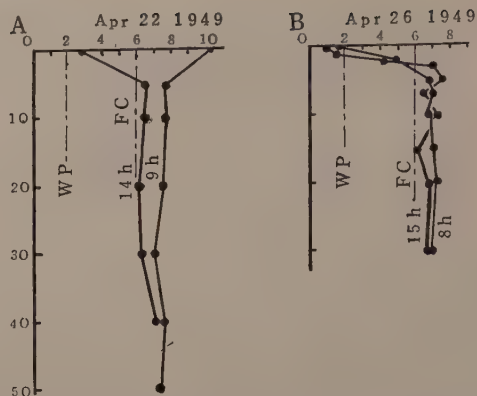


Fig. 10. Vertical distribution of soil water (Absc., %) in the morning and afternoon of the same day in stands. A. Following day after rainfall, B. 5 days after rainfall. Ord. depth in cm.

seen in the vertical distribution within a day. From these facts it will be concluded that the water content of surface soil increased for a while after rainfall but that it was followed by the rapid

decrease in the same layer, after which the variation became extremely slow.

The complete patterns of the vertical distribution of soil water were as follows [25]. During and immediately after rainfall the water content of surface soil far exceeded FC, while the lower layer retained water content near to, but somewhat larger than FC. From a certain depth downwards, i.e., 50 cm. above ground water level, the water content suddenly increased and attained MC at just above the ground water level (capillary water zone). From the surface soil to the upper end of this zone water content was kept in principle at FC (air-containing capillary water zone). By high ground water level such as in marshy places the capillary water zone extends to soil surface, the total root layer belonging to this zone. In our dune soil, however, this case was rather the exception and in most cases the ground water level was deeper than 150 cm., the root layer consequently lying in the air-containing capillary water zone. The most common pattern of vertical distribution was observed during rainless but not too dry conditions in which water content of air-containing capillary water zone was kept at the constant value of FC save the surface layer, this pattern persisting through autumn, winter and spring. In dry season in summer, however, the water content fell short of FC down to 50 cm. depth and approached to WP down to considerable depth, e.g., in the driest condition the soil retained only a water content of about WP indeed at 20 cm. depth. There existed considerable fluctuation of ground water level at various seasons of an year in the lowland between two dunes occupied by pine stands. Ground water level fluctuated with the distribu-

tion of precipitation, having two peaks in June and September and a minimum height in winter. The level in summer during dry period was similar to that of spring. Evidently the ground water level at the top of front dune should be far lower. A hole was dug down to 3.5 m. from soil surface in this place but we could in vain reach to capillary water zone.

Water content in springtime has much to do with the germination of pine seeds as will be mentioned in details afterwards. Vertical distribution of soil water content was measured at pine stands, Plot 9, in the springtime of 1950. As a check the vertical distribution of soil water content at *Carex Kobomugi* Comm. in front dune was added (Fig. 11). On a few days after rainfall upper

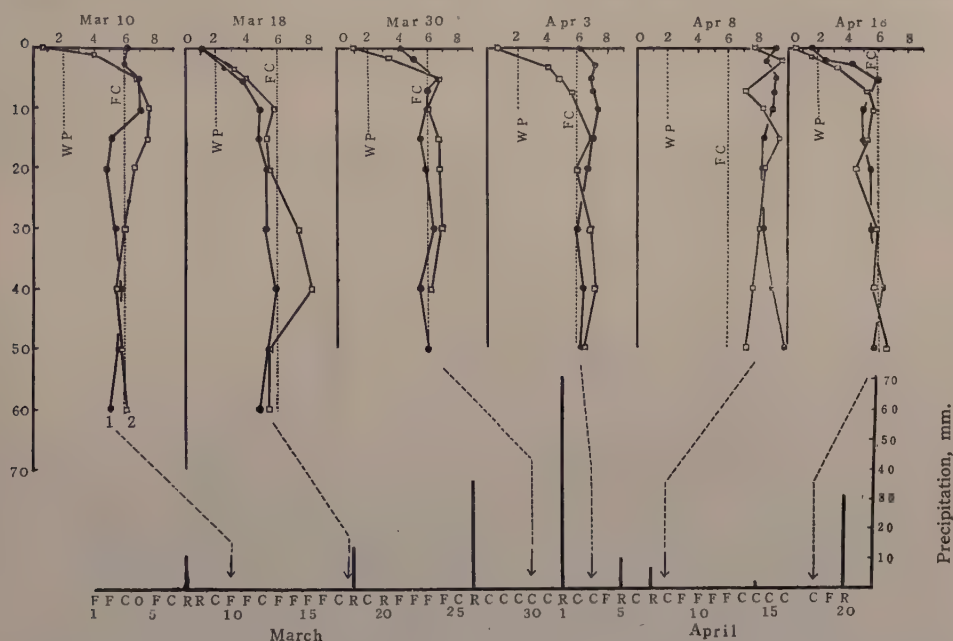


Fig. 11. Vertical distribution of soil water in the spring of 1950 at pine stands (Plot 9) of Chigasaki region. Precipitation amount was measured by a ombrometer in the stands. F, fine day; C, cloudy day; R, rainy day. 1, Pine stands (Plot 9); 2, *Carex Kobomugi*, Comm. at front dune. Ord. depth, Absc. water content, %.

soil retained FC in pine stands (Mar. 10 and Apr. 3), while the surface layer of front dune was much dried. This was due to the thin deposit of dissolved organic substances in pine stands by which the soil surface was tightened to some extent. But during rainless days far from the previous rainfall the surface layer was also dried in pine stands (Mar. 18, Mar. 30 and Apr. 18). The water content of subsoil deeper than 10 cm. was kept at FC save the day immediately after rainfall (Apr. 8). It has been confirmed from Fig. 11 and other measurements that the water content of surface 2 cm. occasionally fell below WP and that we are not in a position to be concerned about the diminished water absorption of pine seedlings germinated in the previous year as well as well as in the



year in question, as roots attained usually to the depth of more than 5 cm. immediately after germination, but germination itself must have something to do with the surface drying.

In summer the situation was quite different from spring. The long interval of rainless days as contrast to plentiful monthly precipitation was mentioned previously. The record of rainless days during the investigation occurred in July of 1949 for about three weeks, from 8th to 27th. In the measurement of vertical distribution of soil water content in summer, the data at the top of rear dune were added as a check as well as those of front dune (Fig. 12). During the dry period above mentioned, the water content was already below WP to

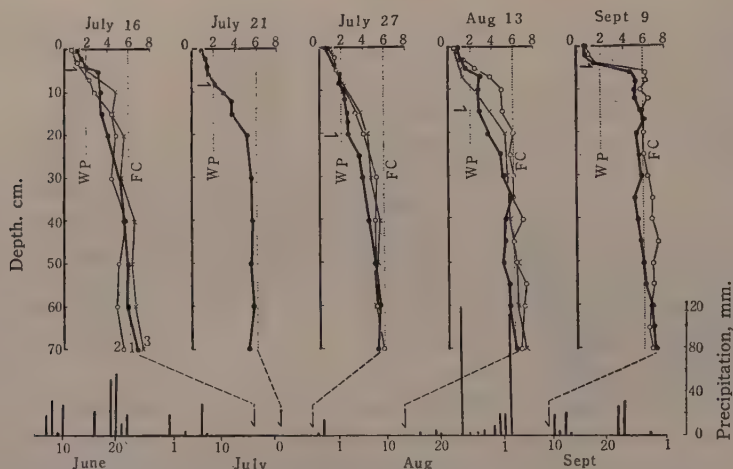


Fig. 12. Vertical distribution of soil water during dry season in the summer of 1949 at pine stands (Plot 9) of Chigasaki region. The allows show the depth below which water content exceeded WP in Plot 9. 1, Pine stands (Plot 9); 2, *Carex Kobomugi* Comm. at front dune; 3, *C. Kobomugi-Imperata* Comm. at rear dune. Absc. water content, %.

the depth of 5 cm. on July 16, nine days after rainfall and the dried part proceeded to nearly 10 cm. depth on July 21, the driest condition in our investigation being observed on July 27 immediately after the beginning of the next rainfall, in which water content 20 cm. below soil surface approached WP. Attention must be paid as to the water condition in dry intervals in that the water content of both dunes scattered with a small number of dune plants was higher for a considerable range of depth except for the surface layer, owing perhaps to the difference of water absorption by roots (July 27, see also Aug. 13). In 1949 the second dry interval came during July 30 and Aug. 15, but surface drying did not proceed so far as the first dry interval. With the advent of September the intervals of rainless days became shortened and only 5 cm. depth of surface soil was dried below WP at the beginning of next rainfall (Sept. 9). In the summer of 1950 the region was visited by two dry intervals, June 29-July 9 and July 14-July 26. The drying pattern was similar as the previous year, but was not

so serious as that of July 27, 1949, only the water content of 12 cm. depth fell short of and approached WP. During autumn and winter the distribution pattern was similar to those of spring and no difficulty seemed to occur as to the survival of pine seedlings concerning, at least, the water relation.

Some measurements, though not so exhaustive, of the vertical distribution of soil water content were also conducted at Arai dune stands during 1950. The measurements were confined to Plot 14 and 15, representative ones of Fukiyose-shita stands, with a few exceptions. These plots are situated at the middle part of the stands, ranging from the top of front dune to that of rear dune. The slopes of both dunes were partly covered by good stands and partly by poor ones, the lowland between the dunes having excellent stands excluding a part in Plot 15. The northern part of the lowland was marshy with sparse main tree-crops (Fig. 13). The soil water content—depth relationships are illustrated in

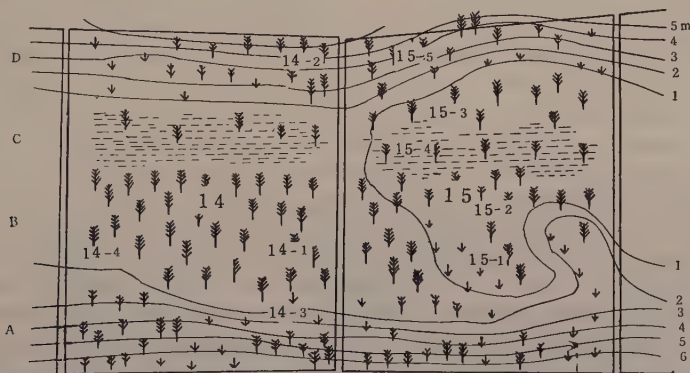


Fig. 13. The outline of Plot 14 and 15 at Fukiyose-shita stand in Arai University Forest in 1950. Each tree-crop was drawn according to size. A, Slope of front dune; B, Lowland; C, Marsh; D, Slope of rear dune.

Fig. 14. From the figure it will be noticed that the FC was smaller than those of Shōnan dune region—a little more than 4% on oven dry basis. The value at the top of rear dune seemed to be somewhat larger, but it might be too early to give the conclusion by such a few data. The ground water level at the excellent stands between two dunes became higher as the proceeding of the growing season till the end of rainy season at the beginning of July and reached to 60 cm. from soil surface on June 30, while the adjacent poor stands at a little elevated places of Plot 15 as well as in other plots have always tree-crops with flat root system lacking in taproot. But this situation could not be applied to the slope of both dunes in which good stands were frequently seen notwithstanding deep ground water level. Most of the measurements of this region were done on days not far away from the previous rainfall and the driest condition, with chagrin, could not be caught during the stay in this region, but it was some slight consolation of the author to be able to measure the vertical distribution on Sept. 1, 1950, two days after the previous rainfall. Before this date, however, rainless days had presumably continued more than a week as could be supposed from the

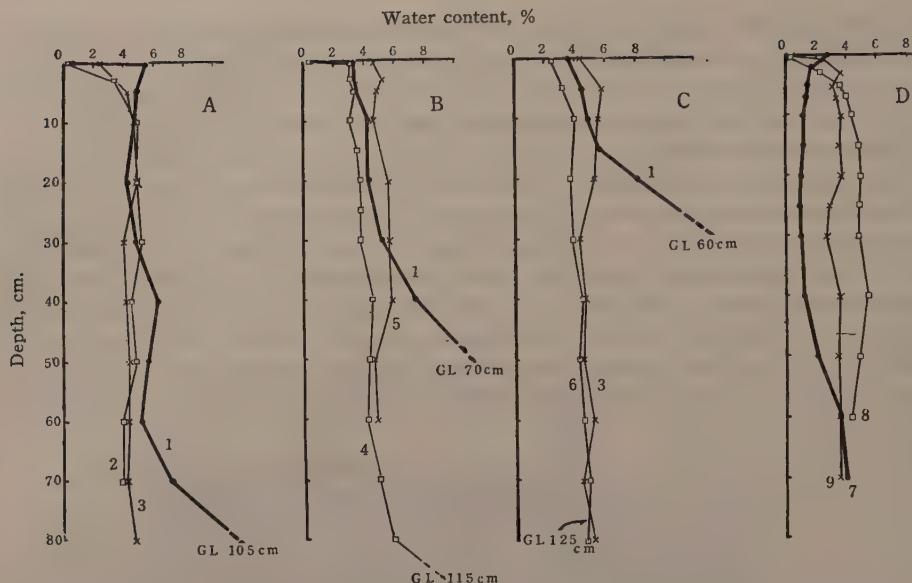


Fig. 14. Several data on the vertical distribution of soil water in Arai University Forest. A, Mar. 18, 1950; B, May 7, 1950; C, June 30, 1950; D, Sep. 1, 1950; GL, ground water level. 1, Plot 14-1 Excellent stands; 2, Plot 15-1 Poor stands; 3, Plot 15-5 Good stands; 4, Plot 12 Poor stands in lowland; 5, Plot 8 at the top of rear dune; 6, Plot 14-3 Poor stands; 7, Plot 5 Excellent stands at the slope of front dune; 8, Plot 5 Poor stands at lowland; 9, Plot 5 Good stands at the slope of rear dune.

precipitation data of Shōnan dune region. In this day the vertical distribution of soil water content was determined at three places at the west part of Fuki-yoseshita stand (Plot 5), a similar situation with Plot 14 and 15. In excellent stands at the north slope of front dune the water content of the surface soil was somewhat larger than WP, owing perhaps to the previous rainfall, while the water content of lower parts was kept near to WP indeed down to 40 cm. under soil surface, showing that the effect of the previous rainfall only halted at the surface soil due to canopy interception. From these results it was revealed that the surface drying of this region far exceeded those of Shōnan region, in which only 20 cm. of surface drying below WP was observed in the driest condition. Interestingly enough was the water amount of poor stands between the two dunes showing far larger water content at 5 cm. under soil surface, which may indicate the minor role of canopy interception in this place. The same situation was again substantiated in good, but not excellent stands at the south slope of rear dune.

The above mentioned results are only comparable with the report of Kadota [6] in his study of the relationships between soil water content and precipitation in Shōnan dune region. By his results the surface dryness reached only to 10 cm. depth or thereabouts at the driest period. But his measurement was done, conceivably, at new afforested parts near the top of the front dune, in which the author obtained the same results, and if he had measured the soil water content of pine



stands inside the front dune, he would have found the far reaching dry conditions as were measured by the author.

#### IV. GERMINATION OF PINE SEEDS IN SPRING

Bearing in mind the water condition of the soil mentioned in the previous section, let us turn to the growth of pine yearlings in their habitat. Vast number of pine seeds fell to the ground in late autumn and germinated simultaneously in the next spring, out of which only a negligible number survived throughout the year. The seeds fallen to the ground were at first attacked by birds and considerable numbers were depleted. Those seeds escaped from bird's attack were exposed to alternately dry and wet conditions of surface soil in the germinating season. So the success or failure in germination may firstly depend upon the soil water relations surrounding them.

The relation between germination rate and soil water content was experimentally studied for the purpose of finding out the lower limit of water content for germination in the habitat. At first the seeds were dipped into the soil of Chigasaki dune region with definite water content and the possibility of germination was examined. Respectively 10 seeds were sown in glass tubes with elevated soil water content, from 2.4 to 24.0% on oven dry basis on June 24, 1949 (Table 9). On July 4, germination had been observed in all water content save 2.4% and on July 6 seedlings came out evenly at water content above 3.7%, no signi-

TABLE 9

The relation between soil water content and germination of pine seeds.  
10 seeds were sown in each glass tube on June 24, 1949

No.	Soil water content on June 24	Germination number on July		The condition on July 8		
				Fresh weight for individual	Soil water content	
		4th	6th		Upper	Lower
1	2.4%	0	0	—mg.	2.17%	2.51%
2	2.4	0	0	—		
3	3.7	2	5	47	2.98	3.22
4	3.7	3	5	50		
5	4.1	2	6	45	3.11	3.35
6	4.1	4	8	57		
7	5.1	2	5	74	4.72	4.84
8	5.1	4	7	50		
9	6.4	2	8	58	4.72	5.03
10	6.4	3	7	53		
11	7.2	3	6	67	6.24	6.53
12	7.2	3	9	64		
13	24.0	5	7	73	18.2	23.5
14	24.0	2	9	57		

ificant difference was observed in fresh weight of seedlings at each water content. At that day soil water content had been diminished to a considerable degree owing to water absorption of seeds and evaporation. A similar experiment was carried out on the spring of 1950 lying stress on the water content around the threshold for germination. Germination totally failed between the water content of 2.0 and 3.2, while considerable germination was observed above 3.5%. The same sort of experiment was repeated in October, 1949 with perfectly similar results [24]. In those experiments the weight of not germinated seeds in soil water content of 3.2% had already attained 1.25 times of that at the beginning of the experiment. Thus, following experiment was conducted to make clear the water content of seeds at germination and the process of water absorption. 30 seeds were buried in the soil of their habitat (150 g. of dry weight) in specimen bottles. The water content of the soil was set up in definite values. The seeds were daily taken out from the bottle, weighed after sifting the seeds

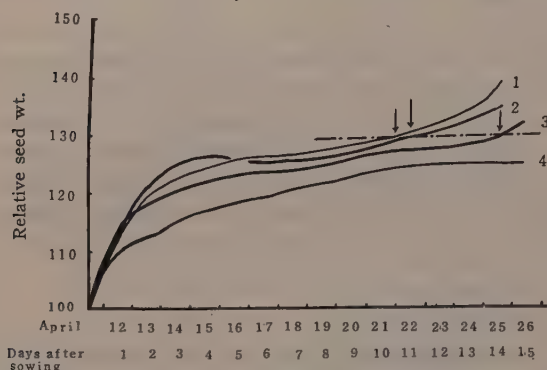


Fig. 15. Water absorption process of pine seeds by germination, sown on April 11, 1950 on moist filter paper (1) and in soil with the water content of 6.6% (2), 3.2% (3) and 2.2% (4). 30 seeds were, respectively buried in soil (Dry weight 150 g.) with elevated water content. The allows show the relative fresh weight at germination, putting initial weight as 100.

from soil, and were again buried in soil with the same water content as before. By this procedure the variation could be caught of seed weight due to water absorption in germinated as well as in not germinated ones (Fig. 15). At soil water content in the neighbourhood of FC (6.6%) the process of water absorption was similar to the seeds sown on a moist filter paper. The seeds also germinated at the water content of 3.2% but the germination was delayed a few days in comparison with two cases above mentioned, while no germination was observed at the water content of 2.2% (near to WP), though the seeds had absorbed considerable amount of water at the end of the experiment. Interestingly enough was the conformance of seed weight, 1.28 times of the initial weight in three successful cases for germination at the emergence of root tips from seed coats. After germination the weight of seeds rapidly increased. To sum up, the germination will occur above the soil water content of 3% provided that the seeds were surrounded by constant water content. In the upper layer of dune soil in their habitat, however, the seeds are exposed to alternate drying and wetting during germination. From this it may be quite natural to suppose that the seeds are often fallen into a dry condition just after germination when their root length is not long enough to absorb water from lower layer of soil with plentiful available water. In this circumstance the drought resistance of germinating seeds may have much to do with the survival. An experiment was designed in which just

germinated seeds with root length of below 0.5 cm. were put in dry soil for definite intervals, after which they were buried again in wet soil, the vitality being examined by their subsequent growth (Fig. 16). After several days in dry soil the fresh weight diminished to a considerable extent, but the vitality was only slightly lost. The drought resistance of germinating seeds, therefore, seems to be comparatively high.

Based on the experiments above mentioned, let us discuss the actual germination in their habitat. The drying process of surface soil during the germinating season of April was illustrated in Fig. 17. At the depth of 4 cm. under soil surface in pine stands of Chigasaki region, the soil water content was kept, after eleven rainless days, slightly above 3%, the lower limit of water content for allowing seed germination as was mentioned above. But this long interval of rainless days was rather an exceptional case

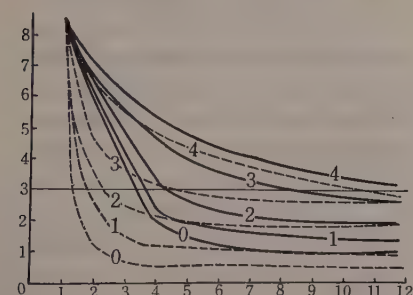


Fig. 17. The drying process of surface soil in germinating season at Chigasaki region. Solid and broken lines are the process, respectively, in pine stands and in front dune. The figures, 0-4 show the depth in cm. under soil surface. Ord. water content, %; Absc. days after rain.

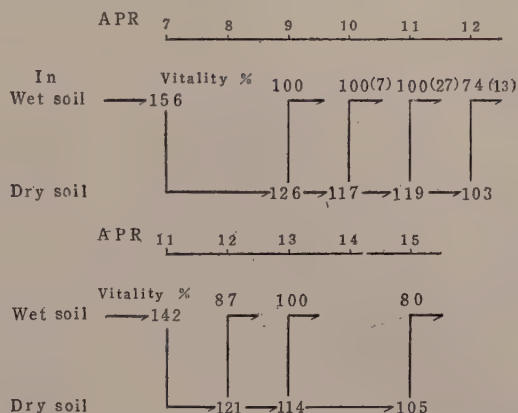


Fig. 16. Drought resistance of germinating pine seeds in the spring of 1950. Seeds were germinated in wet soil and then removed to dry soil. After definite interval they were again put in wet soil and the vitality was tested by their subsequent growth. The figures on line are the index of seed weight, the weight at sowing as 100. Vitality was shown as %. The figures in parenthesis are the percentage of seeds killed after germination. The root length was already 0.2-0.5 cm. when seeds were removed to dry soil.

and this region was visited by 4 or 5 times of 4-5 rainless days as was made clear by the measurement of precipitation in the April of 1949 and 1950 (See Fig. 11). In *Carex Kobomugi* Comm. at the top of front dune, however, the water content fell below 3% down to 2 cm. below soil surface, only about two days after rainfall, and 3% of water content was scarcely kept even at the depth of 3 cm. under above mentioned rain type. So, during 5 rainless days the germinating seeds lying 0-2 cm. below soil surface may be put in dry condition (below 3% of water content) for two or three days. Judging from the decrease in fresh weight of germinated seeds put in dry soil (Fig. 16) and the water absorption process of germinating seeds (Fig. 15),

great difficulty for germination can easily be anticipated from the water relation



of the soil, for, putting the fresh weight of dry seeds as 100, the fresh weight of germinated seeds fell from 150 to 115 after 3 days in dry sand and from the seed weight of 115 it took 9 days for the seeds to attain the fresh weight of 128 and to succeed in germination even in the best water condition of soil, though bearing in mind the different situation of 'germinating' and 'germinated' seeds this interval may be shortened to some extent. Anyhow, if germinated seeds once dried begin to absorb water after rainfall there may be a great possibility of being confronted with next rainless days. Repeating water absorption and water loss in accordance with the fluctuation of soil water content the vitality will be gradually lost and eventually the seeds will be totally perished. From above the upper limit of seed position to germinate against soil-surface drying may be 2 cm. or thereabout under soil surface. In the soil of pine stands, e.g., Plot 9, the germinating condition was somewhat ameliorated. In this case the water content of surface soil had just been reduced to 3% even after 4 or 5 rainless days, so it seems that all seeds will be able to germinate except those exposed on soil surface. But it must be remembered that the measurement of soil-water content was conducted at the place with settled soil surface. The germination of pine seeds could hardly be observed in such places, owing perhaps to the impossible burying of seeds, which may facilitate bird's attack and drying of the seeds. In

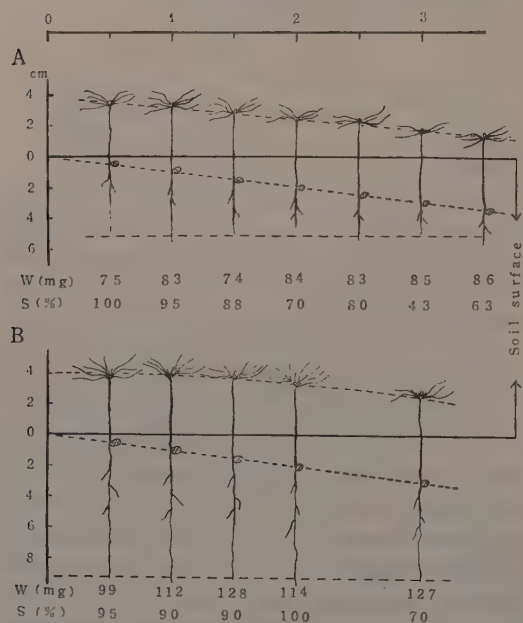


Fig. 18. The forms of seedlings germinated from different depth of soil, measured and sketched on A) Apr. 28, 1950 (Sown on Mar. 8) and B) July 11, 1949 (Sown on June 13). W (mg.) is the weight of a seedling at measurement and S (%) is the survival rate. Absc. seed depth, cm.

pine stands the yearlings were found during germinating season in places trampled down by fallen leaves gathering during winter. At the time of the investigation the post-war shortage in fuels necessitated the fallen leaves gathering. The soil surface of the trampled down places was not settled and the drying process of surface soil resembled rather that of the front dune, the germination of seeds near to soil surface, consequently, seems to be hardly possible.

So far, the upper limit of seed depth in the soil was considered from surface drying of the soil. From soil water relations the deeper the seeds lie, the better the condition for germination. But another factor for limiting the survival of yearlings must be the emergence of cotyledons out of the soil, because even if the seeds germinated at a considerable depth the seedlings will be sooner or

later killed if reserve substances of seeds were exhausted before cotyledons emerge out of the soil. From this nutrient problem it is tempting to consider the lower limit of seed depth for the survival of the seedlings. Also this problem was experimentally studied by sowing respectively 10 seeds in different depth in glass tubes filled with dune soil whose water content was prepared and maintained in the neighbourhood of FC, the experiments being carried out in spring, summer and autumn of 1949 and 1950, from which two examples were selected and illustrated in Fig. 18. In every experiment the lower limit of seed depth for obtaining pine seedlings seemed to be at 3.0–3.5 cm. under soil surface. Interestingly enough was the form of seedlings germinated from different depth in that their stem length was nearly the same, while the root length became shorter as the depth of seed position. The height of yearlings, consequently, decreased as the seed position became deep, but the depth of root tips was nearly constant.

Thus, for the successful germination and survival of yearlings in spring time the seeds must escape from two limitations, i.e., 1) the impossible germination by soil surface drying and 2) the impossible coming out of the soil surface due to nutrient relation, the possible survival may only be confined to seeds lying from 2 to 3.5 cm. under soil surface. The direct observation of germination in their habitat substantiated these inferences (Table 10).

TABLE 10

The distribution of seed depth at germination of pine yearlings found in pine stands (Plot 9) of Chigasaki region surveyed on April 30, 1950

Seed depth (cm. from soil surface)	0.0	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4.0	4.5
% of distribution	0	0	3	7	23	35	28	5	0	0

In the pine stands of Arai dune region scarcely any germination was seen in spring, the first seedlings appearing only during the rainy season of June, owing perhaps to worse soil water relation. In this case it may be that the soil surface drying in spring is so severe that the two limitation depths overlap each other and there remained no depth for the seeds to be able to germinate and emerge out of the soil. After all the yearlings in pine stands of dune regions are doomed to be subjected to severe trials from their initial stage of growth, i.e., germination.

## V. THE FATE OF PINE YEARLINGS IN SUMMER

After the trial at germination in spring, pine yearlings in their habitat enjoyed life and grew rapidly during May and June, knowing not, perhaps, this good luck was only a momentary one before fatal tragedy in summer, in other words, the yearlings were destined to be confronted, like or dislike, with summer drought in near future. In this connexion, therefore, we are driven by necessity to make clear the drought resistance of pine yearlings before facing the most cardinal problems of pine yearlings in their habitat during the fatal days. We shall begin by making clear the water economy of pine yearlings at the

emergency of their water relations.

The most general expression for the water economy of plants is

$$W = W_t - W_0 = \int_0^t A dt - \int_0^t T dt, \quad (1)$$

where  $W_0$  and  $W_t$  are the water amount at the beginning and after  $t$  hours,  $T$  and  $A$  are transpiration and water absorption amount of whole plant or plant parts for unit time. As was referred previously, we can never measure the amount of  $A$  directly except for water-cultured and autoirrigated plants. So we are obliged to approach the problem of water economy from water amount and transpiration.

At first the transpiration was measured of shoots for the yearlings sown on April 1, 1950 and raised in the soil of their habitat at glass house of the Faculty of Science, Tokyo University. The shoots of yearlings were hung to a torsion balance immediately after detaching with cut ends sealed with vaselin, the decrease in weight of yearlings being measured at definite intervals. The torsion balance was put in a glass-sided box in order to avoid the movement of the air and the saturation deficit in mm. Hg. was determined by a psychrometer put in the neighbourhood of pine shoots, by which the obtained transpiration amount was converted into mg./g. dry weight/10 mm. Hg./hr. for comparison's sake.

The chief experiments were conducted in the summer of 1950 when primary

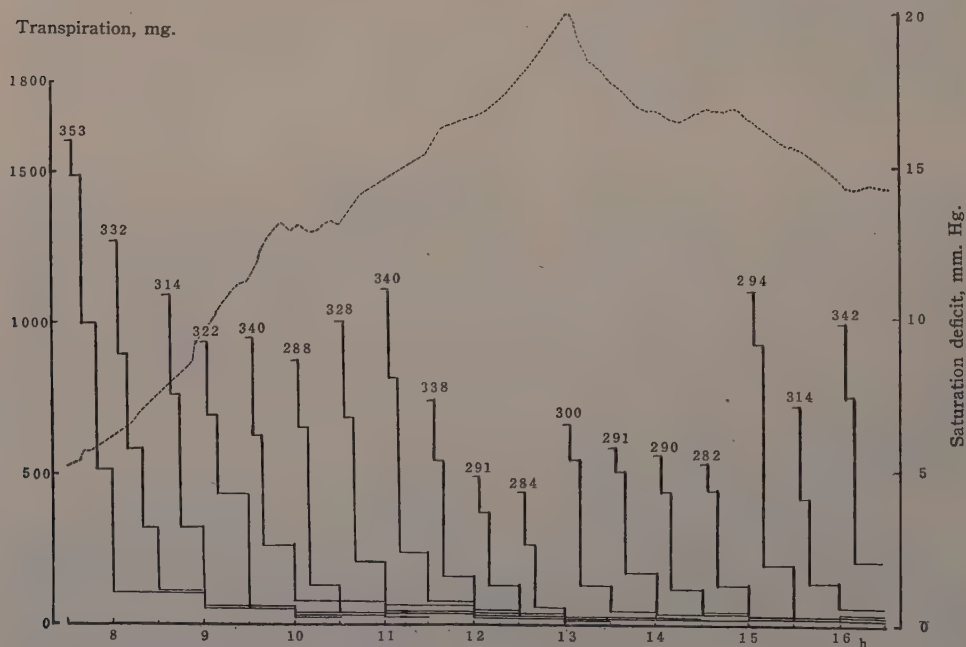


Fig. 19. The variations of transpiration of pine yearlings detached at different hours of day on July 14, 1950. The figures at the tops of each curve are the water content on oven dry basis of yearlings immediately after detaching. Dotted line is saturation deficit, mm. Hg.



leaves were 1.5 cm. long. As an example the result of an experiment was illustrated of a fine day on July 14 when the soil had yet sufficient available water (Fig. 19). From 7.30 h. to 16.00 h. the shoots were successively cut every 30 min. and the process of transpiration after detaching was measured respectively. The amount of transpiration fell, without exception, to such a low amount as 20 mg./g. dry weight/10 mm. Hg./hr. after 30 min. from detaching and this value persisted for a long time. The final value of transpiration seemed to have no bearing on the time of detaching and this value persisted for a long time, whereas that of immediately after cutting was the highest in the morning, decreased to a considerable extent in the afternoon and again increased somewhat in the evening, owing perhaps to the daily march of stomatal movement. The xerophytic character of Japanese Black Pine (*Pinus Thunbergii*) was shown in that the final transpiration amounts were only between 1/80 and 1/20 of the initial value even at the herbaceous stage of young seedlings. The daily variation of water content of shoots immediately after detaching followed similar trend as that of transpiration amount at that time.

As the water content of the soil diminishes to the range of WP, water absorption of plants practically stops, so the first term of equation (1) is negligible and the equation will be,

$$W_0 - W_t = \int_0^t T dt, \quad (2)$$

and in this case we are able to examine the water economy of drought resistance by water content and transpiration. Detailed analysis by equation (2) of the dehydration resistance of pine yearlings was written in another paper [26], as the analysis has directly little bearing on the fate of pine yearlings in summer. Suffice it for the present position to solve the problem by water content and cuticular transpiration, for the stomata were already closed when the yearlings were threatened by drought.

An experiment was set up in which the water supply of a pot was cut off from July 19 to July 25 and the process of withering was followed of pine yearlings during soil water exhaustion. At first the process of transpiration decrease after detaching shoots was measured several times a day. On July 19 and 20 the trend was similar to the control (Fig. 19), whereas abrupt decrease of initial transpiration set in on July 21, the maximum value immediately after detaching the shoots being only 1/3 of those in previous days. From the evening of July 21 transpiration amount showed, from the beginning, extremely small values similar to those after long intervals from detaching in the case of yearlings with normal water supply, these amounts remaining unchanged for a long while after detaching (Fig. 20). The amount of transpiration, 10–20 mg./g. dry weight/10 mm. Hg./hr., coincided in both cases of crisis in water economy, i.e., those through detaching shoots and by the exhaustion of soil water. Similar experiments were also carried out at their habitat in that summer using yearlings sown on March 2, 1950 directly at Plot 9 of pine stands in Chigasaki region. The measurement on July 9 was illustrated in Fig. 21. The amount of transpiration immediately after detaching was already decreased to such an extent as that of July 21 in the previous experiment and the final values of transpiration after long intervals

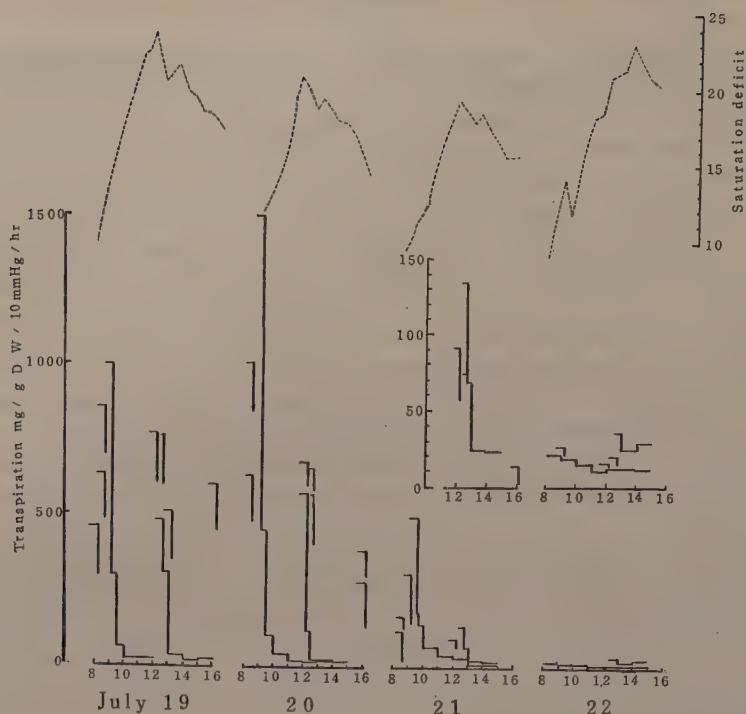


Fig. 20. The variations of transpiration of pine yearling in the drying process of soil. The process of transpiration decrease was shown in two cases a day and others were omitted. The values of July 21 and 22 were enlarged and illustrated at upper part.

from detaching were also the same. Considering from the vertical distribution of soil water on that day when water content was slightly higher than WP down to 15 cm. under soil surface and the root tips of yearlings had attained in average to the same depth, the results are understandable, and it was affirmed that the situation was quite the same in yearlings at their habitat as well as those raised in laboratory. In passing, some results of winter experiments, though not the main point, will be referred. Pine seeds of their habitat were sown on October 3, 1949 and the yearlings were raised throughout the winter, their transpiration amounts were measured in January and February of the next year. The results as a whole conformed well with those of summer except that the transpiration amounts immediately after detaching were remarkably small, i.e., between 100 and 200 mg./g. dry weight/10 mm. Hg./hr. in contrast to over 1000 mg. in the same unit during summer. The results in winter may tell the insufficient opening of stomata even in the daytime.

So far the experimental results of transpiration of pine yearlings were mentioned and the author wishes to concern himself with another component of water economy, i.e., water amount of shoots. Water amount *per se* has no direct bearing on drought resistance, for the plants have yet a considerable amount of water while withering due to dehydration, so this lethal water amount must be deduced from total water amount in order to determine the 'verfügbares' [26]

water amount till the death by drought, i.e., lethal deficit (= water amount—lethal water amount). For the determination of lethal deficit lethal water content must be determined at first. The vitality of tissues or organs is often examined either by plasmometric method making most of the failure in plasmolysis in hypertonic solutions due to the change of permeability of protoplasmic layer at the time of withering, or by the activity of catalase or peroxidase which can be detected by the colour reactions with benzidine, pyrogallol, resorcinol or hydroquinone. These methods could hardly be applied to our pine yearlings with their extremely small size. Pisek [16] in his paper concern-

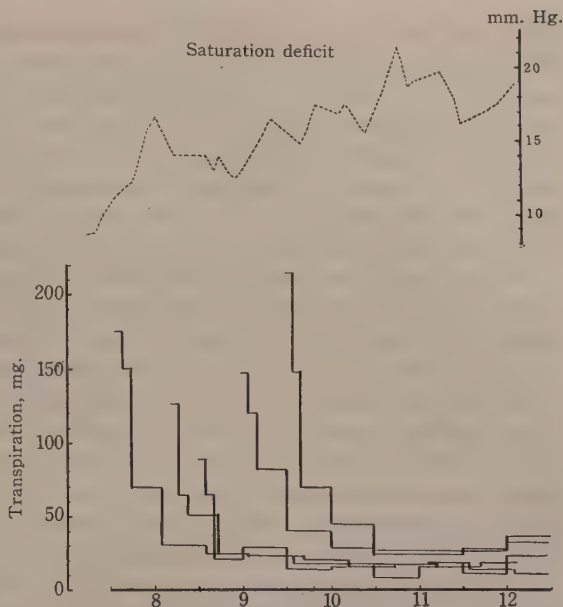


Fig. 21. The variations of transpiration of pine yearlings after detaching them from their habitat at pine stands of Chigasaki region during the drought condition of 1950. July 9. Absc. hours of day.

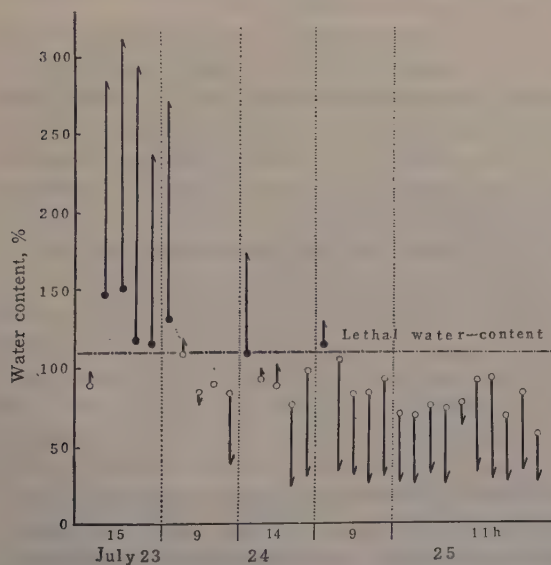


Fig. 22. Determination of lethal water content in pine yearlings. Black and white dots are the water content of living and dead shoots at soaking plants in water. The arrows show the variations of water content after soaking for 24 hrs.

ing "Trockenresistenz isolierter Blätter und Sprossen" determined lethal water content by soaking the petiole of detached leaves, examining their vitality from the success or failure in the recovery of turgescence state. Taking the hints from this treatment the author soaked in water the roots of intact yearlings instead of the base of detached shoots, as water absorption in the latter case is often hampered by resin or mucilage overflowing at the cut ends. Then the variation of their water content 24 hrs. after soaking was determined by measuring the fresh weight at the beginning and 24 hrs. after soaking, but the former could not be determined directly and was obliged to be calculated



from the fresh weight of shoots indirectly estimated from the fresh weight of whole plants and the top-root ratios of yearlings in the same condition, as roots were not cut at that time. The result was that living shoots absorbed vividly water after soaking, while the water content of dead shoots remained unchanged or even decreased in most cases. This different behaviour in relation to their variation in water content was so apparent that the value of lethal water content, 110% on oven dry basis, could easily be determined (Fig. 22).

Simultaneously with the variation of transpiration amount during the course of withering of pine yearlings subjected to drought condition (Fig. 20), the water content was measured of soil as well as of yearling's shoots and was illustrated together in Fig. 23. The weather was fine from July 19 to 25 with extremely high temperature of 30-36° in glass house during the daytime. Soil water content at the bottom of pots was determined once or twice a day, which was 7.6% (a little above FC) on July 19, decreased to 2.3% (near to WP) in the afternoon of July 21 and fell below WP after July 22. It was of interest that both transpiration amount and water content of shoots were kept in almost normal condition so far as soil water content was above WP but abruptly began to decrease when the latter approached WP. As the water content of soil in shallower parts was always smaller than that in the bottom part, it may be concluded that the water economy of pine yearlings remained normal so far as there existed some available water adjacent to their root layer. The water content of shoots fell below lethal water content and the yearlings began to wither only a little more than one day after the soil water content had dropped below WP and after two days nearly all yearlings were gone.

The environmental condition of glass house with maximum temperature and saturation deficit, 36° and 23 mm. Hg. respectively, may seem, at first sight, to be an extraordinary case, but that this condition was not an uncommon case prevailing in their habitat may be revealed from the saturation deficit measured in their habitat on a fine summer day (Fig. 21). Besides, wind may produce more severe evaporating condition, thus hastening the dehydration of yearlings to a certain extent. Field observations on the withering of pine yearlings done during the drought season in summer of 1949 (Fig. 12) substantiated the results of above mentioned experiment. Thus in that year rainless days continued for 20 days, from July 8 to 27. On July 16 the soil water content was higher than WP from the depth of 4 cm. downwards, then the yearlings germinated in spring were all living, while on July 21 drying to WP proceeded down to 8 cm. under soil surface accompanied by the withering of poor yearlings with root tips within 7 cm. depth. In July 27, just before the next rainfall the drying proceeded to 20 cm. under soil surface and most of the yearlings had been killed by that time save those which had grown so excellently that their root tips had attained to the depth of more than 20 cm. under soil surface. Lastly, some simple calculations will be added of the relationship between lethal deficit and transpiration during drought condition, though the details will be left to another paper [26]. The water content at stomatal closure in drought condition was 280% on oven dry basis. Then, lethal deficit will be 170%, obtained by deducing lethal water content, i.e., 110%, from 280%. So, 1 g. dry weight of shoots has 1700 mg. of

'verfügbares' water until death by dehydration. On the other hand it took 48 hrs. for the water content to decrease from 280 to 110% (Fig. 23). Thus the yearlings

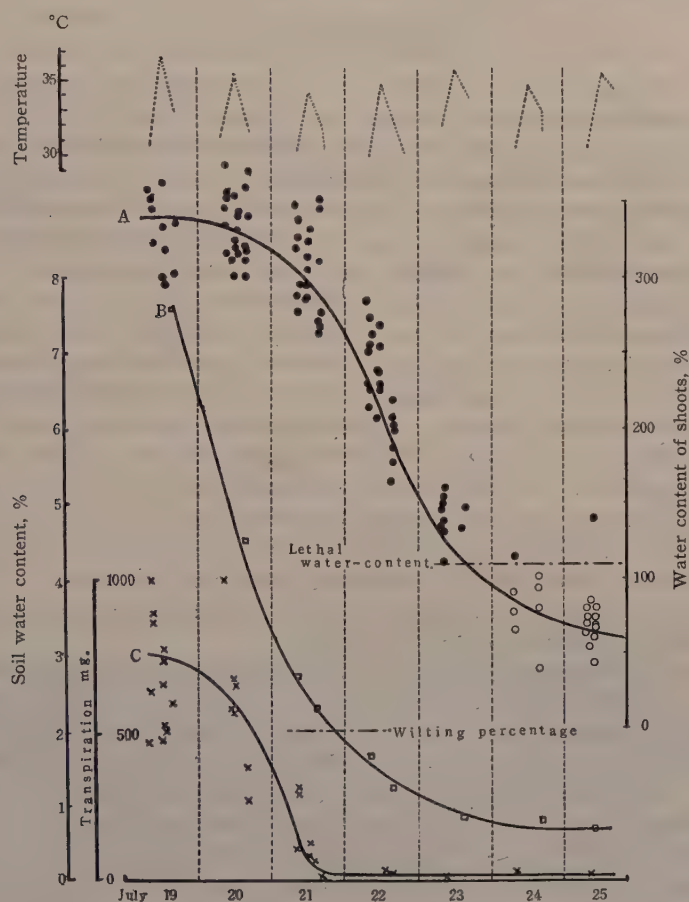


Fig. 23. The variations of A) water content of shoots, B) water content of soil and C) transpiration amount (mg./g. dry weight/10 mm. Hg./hr.) immediately after detaching shoots, in accordance with the process of soil drying. The yearlings were raised from the seeds sown on Apr. 3, 1950 in pots filled with the soil of Chigasaki dune region.

had transpired in average at the rate of 35 mg./g. dry weight/hr. if the water content of roots can be disregarded. Meanwhile the transpiration rate measured during this condition was 20 mg./g./10 mm. Hg./hr. Considering that the saturation deficit during the day time in cultured condition was over 20 mm. Hg., the calculated rate of 35 mg. is understandable.

In conclusion, both experiment and field observation told us that the survival of the yearlings in summer has much bearing on the depth of root tips attain-

able till summer drought to the depth under soil surface at which water content is constantly kept above wilting percentage.

### CONCLUSION

So far, the fate of pine yearlings in dune regions was investigated with special reference to their water economy and it was affirmed that the fatal condition was the shortage of available water in the soil and that the root length was the decisive factor for the survival of yearlings. A few seedlings found in the habitat, therefore, may be those seedlings which had endured the summer drought of the first year with exceptional good growth of roots. The problem of the fate of pine yearlings in dune regions has silvicultural importance as well as ecological interest, as the conventional method of wind-break rearing with planting pine seedlings more than two year old often ends in a failure making poor tree-crops without taproot as was often observed in two dune regions, especially in Arai stands, while a few seedlings grown from seeds in those regions showed excellent growth without exception. Important must be the root depth of yearlings long enough to be able to endure the summer drought for raising pine stands from seedlings. For this purpose autumn seeding will be advisable, for increased root depth in autumn sown yearlings can be expected as the seeds are sown 6 months earlier than spring sowing. The experiment of autumn sowing was in progress in dune region of Chigasaki during the winter of 1950-1951, but unfortunately this was obliged to be abandoned owing to the occupation by parade ground of the experimental plot in that year.

Water, as an environmental factor, has been considered to become seldom the limiting factor for the growth of wild and cultivated plants in Japan as the amount of precipitation is plentiful and even in our country. But by the peculiar rain type in summer of Pacific coast of Japan, the universality of drought condition may be anticipated. In fact, drought conditions, though not so serious as the case of our pine yearlings, were observed in some other cultivated plants, e.g., the investigation of Satoo [18] in seedlings of some conifers raised in the soil of Kantô loam with such a large available water capacity (Fig. 4). This sort of drought condition may be numerous in Pacific Coast of Japan, though little attention has been paid to it up to the present. In this case the second terminology of drought resistance of Stocker [19] can be applied and the yield in drought conditions must be investigated from various point of view, which awaits future study.

### SUMMARY

The growth of pine yearlings (*Pinus Thunbergii*) in coastal dune regions was studied both by laboratory experiment and by field observation. The field observation was done chiefly in Shônan region, Kanagawa Prefecture, a part of which was also done in Arai region, Shizuoka Prefecture. Field observations were focussed especially on *Pinus-Imperata-Lespedeza* Comm. of Chigasaki region, Shônan dune.



1. The chlorine content in soils of both dunes was in average 1 mg. Cl/100 g. dry soil and chlorine factor *per se* can not be the limiting factor for the growth of yearlings, as they can endure the chlorine content of more than 10 mg. in the same unit. The amount of total-N and other forms of nitrogenous compounds differed to a great extent according to various site, but nitrogen factor also lacked qualification as an limiting factor.

2. During rainfall greater part of rain water was lost by canopy interception in light rain and by percolation by the heavy kind.

3. Maximum water capacity (MC), field water capacity (FC), wilting percentage (WP), available water capacity (AWC) and volume weight ( $\rho$ ) of the soils of both dunes were as follows:

Region	MC	FC	WP	AWC	$\rho$
		oven dry basis, %			
Shōnan	30	6	2	4	1.5
Arai	28	4	1	3	1.5

These constants related to water condition belong to one of the smallest values ever studied.

4. In the soil of pine stands in Chigasaki region the soil water is lost by soil surface evaporation and transpiration, but daily amount of water loss in midsummer during fine days was estimated to be only 1 mm. or thereabouts in the unit of precipitation, as soil surface evaporation abruptly diminished with the drying of soil surface and the standing crop of undergrowth was comparatively small.

5. The drying of soil surface was not so serious except in summer. Only surface 2 cm. or thereabouts was dried in spring, whereas in summer the drying below WP attained a maximum, 20 cm. under soil surface due to the long intervals of rainless days and severe evaporating condition. Except for surface layer the soil retained water content near to FC till 50 cm. above ground water level, from this depth downward the water content abruptly increased and reached MC at just above the ground water level. Surface 30 cm. belonged to root layer of pine trees and undergrowth and this layer retained only 18 mm. of available water at its maximum, i.e., when the water content was kept FC for all range of root layer.

6. In spring the germination was hampered by soil drying of the surface layer and only the seeds buried at a definite depth, i.e., 2.0-3.5 cm. could succeed in germination as the seedlings germinated at deeper position can never come out of the soil due to nutrient relation. When soil water content was kept constant the seeds were able to germinated at the water content above 3.2% on oven dry basis. Besides, the germinated seeds could endure several days of dry condition, so the temporal drying in their habitat of germinated seeds may have no serious influence upon their vitality.

7. In summer the shortage of available water played fatal role for the survival of the yearlings. As the available water of their root layer was exhausted and reached near to WP for all range of root layer, transpiration abruptly decreased due to stomatal closure. Nevertheless the water content of the yearlings decreased remarkably and they perished in a few days. This process coin-

cided well with field conditions and laboratory experiments. Only a few yearlings with exceptionally well developed roots could survive throughout this season in their habitat.

8. A method was devised of determining lethal water content for pine yearlings by which lethal deficit, i.e., normal water content—lethal water content could be calculated. Next, some discussions concerning drought resistance of the yearlings were referred to, but the details will be left for another paper.

### ACKNOWLEDGEMENT

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## GROWTH ANALYTICAL STUDIES ON THE ARTIFICIAL COMMUNITIES OF *HELIANTHUS TUBEROSUS* WITH DIFFERENT DENSITIES

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### I. INTRODUCTION

The interaction between individuals of the same or different species is one of the most interesting and fundamental problems in the ecology of plant community. The interaction is usually classified into the cooperative and the competitive ones according to the accelerating or inhibiting effects on the growth of individuals in the community. It is the latter, in general, that plays more important role in the development of the community than the former.

Among the studies on the competition of plants, the comprehensive one performed by Clements, Weaver, and Hanson on artificial population of sunflower and wheat is the most famous. They dealt with the correlation between the yield or growth of plants and the environmental factors respecting the density of population. However, they analyzed neither the processes influenced by each of the environmental factors throughout the growing period, nor the difference of the yield or growth in different densities which took place in these processes [4]. Blackman, Watson and others studied the relationships between the yield of field crops and environmental factors including density. In their studies, they made the effective usage of the concepts such as net assimilation rate, leaf area ratio etc. for the analysis of plant growth [1, 5, 22]. Kira et al. studied the relationships between the yield and the density of the communities of field crops, and succeeded in finding some empirical laws fitted well to the density effect observed [10, 17].

Although many studies concerning the density effect provided us with many descriptive results and some empirical laws in the relation of the growth or yield of plants to the density, the studies providing us with the explanation of the mechanism where results such an effect were very few. To analyze this mechanism it is needed to elucidate the relation between the physiological processes such as photosynthesis, respiration etc. which decide the growth directly, and the environmental factors which are changed continuously by the activity and structure of plants and affect contrariwise the growth of plant. On this line, Iwaki discussed the density effect in a population of buckwheat in the light of dry matter production. He also made an analysis of the competition in the mixed population of buckwheat and green grams from the same point of view [6, 7].

In the present study, the authors intend to analyze the growth of the

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artificial community of *Helianthus tuberosus* with different productive structures and different environmental conditions produced by the different spacing plantation on the basis of dry matter production, of which study originated from the perspective conception of Boysen-Jensen [3]. The material used in the present study possesses the tuber with large quantity of reserved substance, making a marked contrast with the seeds used by other investigators [1, 5-8, 10].

The social relations among individuals in a plant community are afforded essentially by the connection of individuals made through the medium changed by the reaction. This is the fundamental principle commonly applicable to either artificial or natural communities. Accordingly, it may be expected that the present study will offer a logical explanation for the social relations of plants not only in the simple and homogeneous community, but also in the complicated natural community.

## II. MATERIAL AND METHODS

As the experimental material, Jerusalem artichoke (*Helianthus tuberosus*) was used for the reason of its vigorous vitality, its strong tolerance to injurious insects and diseases, and its simple growth form.

In the first year experiment the tubers of *Helianthus tuberosus* were planted on March 14, 1956 in a regular square disposition in the experimental field of Tokyo Metropolitan University, Setagaya, Tokyo. Three different grades of spacing, i.e. a spacing of 10, 20, and 30 cm. in both directions, were employed. So the individual number in these plots was 100, 25, and 11.1 per sq. meter, respectively. As the growth in the early stage depends on the weight of tubers, tubers of the same size, 8.3 g. in fresh weight, 1.65 g. in dry weight on the average, were selected. The area of each plot was  $2 \times 2$ ,  $5 \times 5$ , and  $5 \times 5$  m<sup>2</sup>, respectively. The field soil was not fertilized.

In the 1957 experiment the tubers (the average fresh weight 8.3 g., dry weight 1.74 g.) were planted on March 16, in four grades of spacing of 10, 20, 30, and 100 cm. in regular square disposition. The area of these plots was  $5 \times 5$ ,  $8 \times 8$ ,  $10 \times 10$ , and  $10 \times 10$  m<sup>2</sup>, respectively. Prior to the planting, the field soil was fertilized with 0.1 kg. of KCl, 0.9 kg. of  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ , and 0.9 kg. of  $(\text{NH}_4)_2\text{SO}_4$  per sq. meter. The study was obliged to close at the beginning of August owing to some circumstances against the authors' will.

1. *Light factor.* The relative light intensity was determined with two selenium photo-electric cells coupled with a micro-ammeter by a two-way switch. At the measurement in the field one of them was kept horizontally in the open to obtain the full light as the control and the other horizontally placed at various heights above the ground at random in each plot and by means of the two-way switch almost simultaneous readings were taken for each height to obtain an average value. The measurement was generally carried out under the cloudy sky, in order to avoid the large fluctuation of light intensity in the plant community, which resulted from direct sunshine penetration or sun spots in a fine day [12].

2. *Temperature factor.* Air temperature in the community was measured by the Assmann aspiration psychrometer and usual thermometers which had been set at various heights in the community. Soil temperatures at depths of 5, 10, 20, and 30 cm. were occasionally measured by use of soil thermometers. The calculation of dry matter production and of respiration was based on the temperature data of the Tokyo District Meteorological Observatory.

3. *Water factor.* The atmospheric humidity in the community was measured by the Assmann aspiration psychrometer. Soil moisture was determined by the usual gravimetical method. The amount of evaporation from soil surface was estimated from the decrease in weight of plastic dish which was filled with soil with the same moisture and buried at the ground level.

4. *Nutrient factor.* To determine the amount of water-soluble and exchangeable ions, soil extracts for chemical analyses were prepared by the following treatment. Ten grams of the air-dried soil was extracted with 50 ml. of Morgan's universal extracting solution (sodium acetate buffered at pH 4.8) for 30 minutes under a shaking condition.

After digesting the soil extract with  $\text{HNO}_3$  and a small amount of  $\text{H}_2\text{SO}_4$  the phosphorus was measured by the Denigè method. Potassium was determined by the flame photometrical method, nitrate by the phenol disulfonic method, and iron by the dipyriddy method. Calcium was determined either by the Ca-oxalate method or by the EDTA method. Ammonium was determined colorimetrically by adding Nessler's reagent to the sample which was prepared by distillation of the soil extract being added a small amount of  $\text{MgCO}_3$ .

The determination of the salt amount accumulated in plant body was carried out through the chemical analyses on the HCl-extract of plant ashes. The analytical procedures employed here were similar to those mentioned above.

5. *Measurement of plant growth, photosynthesis, and respiration.* The sprouting ratio of ca. 100 percent was observed in the planted tubers. The growth rate in the early stage of development was usually equal in any plot. In the later stage, however, the growth of individuals in the margin of the plot became unequal, especially in denser plots, and it appeared "edge effect". Under these circumstances the sample was taken from the center of the plot avoiding the margin.

In case of measurement of growth, 3-10 individuals were usually taken in each plot to obtain the mean value. Moreover, all individuals in an area of  $50 \times 50 \text{ cm}^2$  were sampled occasionally. To determine the growth, such measures as plant height, fresh and dry weight of leaves, petioles, stems, roots, stolons and tubers, and leaf-area were measured as frequently as possible.

These materials were killed at  $100^\circ\text{C}$  immediately after the sampling, and dried up at  $80^\circ\text{C}$  to the constant weight, in order to determine the dry weight. The leaf-area was determined by measuring the total area of all the leaves of a plant directly or by multiplying the leaf area per unit leaf dry weight by the total leaf dry weight. Of course, it was considered in case of this calculation that the value varies to some extent with leaf ages.

The standing crop of each plot was calculated by multiplying the mean dry



weight of individuals in each plot by their numbers in 1 sq. meter, i.e. 100 in the 10 cm. plot, 25 in the 20 cm., and 11.1 in the 30 cm. ones. To draw up the productive structure of the communities the same procedure as reported by Monsi and Saeki [14] was adopted.

Photosynthesis and respiration of the leaves, stems, and roots were measured with a modified Boysen-Jensen's method in the laboratory at 20° or 25°C under the light or dark condition. Photosynthesis under the field conditions was also measured by the same method as the above mentioned.

### III. RESULTS AND DISCUSSION OF THE EXPERIMENTS

#### 1. *The growth of the individuals in each plot*

The results of the measurement of the growth in plant height, dry weight of an individual plant, dry weight of each organ, leaf area etc., under the condition of various densities are shown in Figs. 1, 2, 4, and 5. As the observed values frequently fluctuate to some extent, the corrected ones read from the smoothed curves in these figures are given in Table 1.

a. *Growth in height.* In the early stage of development when there is no shading of leaves by these of neighboring individuals, there is no significant difference of growth not only in height but also in dry weight in any plot. A little later, simultaneously with the beginning of shading, the growth rate in height becomes larger than that in the former stage without shading. The increase of growth rate, however, does not last long due to the lowering of net production by the shading of the leaves. Therefore, the largest growth in height appeared firstly in the highest density, and then it removed to lower density one after another (Fig. 1, and Table 1).

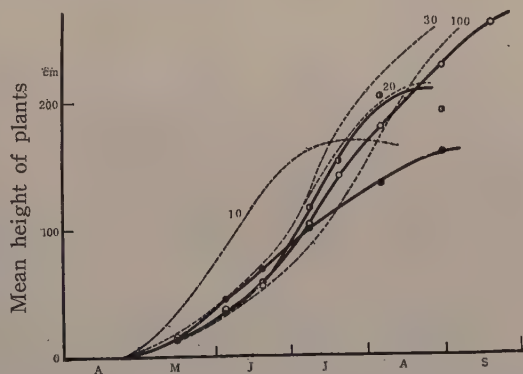


Fig. 1. Growth curve of plant height in cm. Solid lines indicate the curves of 1956 and broken lines 1957 experiment. ●: spacing of 10 cm.; ◐: 20 cm.; ○: 30 cm. Numbers in the figure show the spacing among individuals in 1957.

b. *Growth in the total weight of an individual plant.* It is evident that the total plant dry weight including the planted tuber decreased rapidly till the end of April, in spite of the formation of new leaves, stem, and roots, and that during this period the photosynthesis by the leaves does not play any important role for the growth in the total dry weight (Table 1A). This conclusion is also confirmed by the comparison of the growth of sprouts in the field with that in the dark (Fig. 3). The economic ratio in conversion from reserved substance into active plant organ is considered to be ca. 0.5 as found in other plants such as *Aconitum japonicum*, *Solanum tuberosum* etc. [11].

TABLE 1A  
Average plant height, leaf area, and dry weight of leaves, stem and petioles, roots, stolons, and total individual plant in different spacing plots. A: 1956; B: 1957. Figures are read from the smoothed curves in Figs. 1, 2, 4, 5, 6, and 7.  
The dry weight of planted tuber is shown in parenthesis.

1956												
	March 17	April 14	April 30	May 15	June 4	June 19	July 8	July 20	Aug. 6	Aug. 31	Sept. 20	Oct. 25
10 cm. plot	Height (cm.)	—	—	13.5	44.5	54	99	118	136	—	—	—
	Leaf area (cm <sup>2</sup> .)	—	15.6	64.0	139	290	430	390	—	—	—	—
	Leaf dry weight (g.)	—	0.09	0.32	0.7	1.6	2.2	2.3	2.0	1.4	—	—
	Stem and petiole dry weight (g.)	—	0.03	0.10	0.34	1.0	1.6	2.2	2.7	3.1	—	—
	Root dry weight (g.)	—	0.09	0.24	0.44	0.75	0.83	1.04	1.05	0.89	—	—
	Stolon dry weight (g.)	—	—	0.05	0.36	0.65	0.97	1.06	1.05	1.11	—	—
	Tuber dry weight (g.)	(1.65)	(1.21)	(0.29)	(0.26)	(0.14)	(0.11)	—	—	—	—	—
Total dry weight (g.)	—	0.21	0.71	1.84	4.0	5.6	6.6	6.8	6.5	—	—	
20 cm. plot	Height (cm.)	—	—	—	13	35	58	116	192	192	207	—
	Leaf area (cm <sup>2</sup> .)	—	15.6	64.0	230	550	1110	2250	2640	2640	—	—
	Leaf dry weight (g.)	—	0.09	0.32	1.1	2.7	5.3	8.5	10.2	11.6	10.8	—
	Stem and petiole dry weight (g.)	—	0.03	0.10	0.41	1.3	3.4	7.2	10.7	17.3	25.0	—
	Root dry weight (g.)	—	0.09	0.15	0.37	0.9	1.3	1.8	2.1	2.2	—	—
	Stolon dry weight (g.)	—	—	0.03	0.25	1.0	1.6	2.1	2.3	2.7	—	—
	Tuber dry weight (g.)	(1.65)	(1.21)	(0.31)	(0.20)	(0.16)	—	—	—	—	—	—
Total dry weight (g.)	—	0.21	0.60	2.13	5.9	11.6	19.6	25.3	33.8	—	—	
30 cm. plot	Height (cm.)	—	—	—	13	35	58	105	180	228	262	270
	Leaf area (cm <sup>2</sup> .)	—	15.6	55	230	550	960	2400	4190	4510	4230	—
	Leaf dry weight (g.)	—	0.09	0.32	1.1	3.0	6.0	13.0	18.5	25.5	31.8	12.0
	Stem and petiole dry weight (g.)	—	0.03	0.10	0.43	1.8	5.0	13.5	21.0	31.5	50.0	48.5
	Root dry weight (g.)	—	0.09	0.18	0.62	2.5	3.2	5.3	7.4	17.4	48.5	35.0
	Stolon dry weight (g.)	—	—	—	—	—	1.6	3.3	3.9	9.5	17.4	8.6
	Tuber dry weight (g.)	(1.65)	(1.21)	(0.36)	(0.21)	(0.16)	—	—	—	5.7	3.6	10.2
Total dry weight (g.)	—	0.21	0.60	2.15	7.3	15.8	35.1	50.8	72.4	106.4	108.6	

As mentioned above, the total dry weight of individual plant (oven dry weight) in early stage of development showed no significant difference among three (1956) or among four plots (1957). In the later stage, however, the increase rate of plant weight decreased rapidly under the condition of shading of leaves with those of neighboring individuals, in spite of rapid elongation of stems.

Accordingly, the decrease of growth in total dry weight began primarily in the highest density and lastly in the lowest one. For instance, on April 30, 1956 the mean dry weight of about 1.0 g. per plant was measured in each plot, but on June 19, 1956 the dry weight of 5.9 g. in plant of the 10 cm. plot as compared

TABLE 1B

		1957								
		April 20	May 2	May 16	June 1	June 16	July 2	July 16	Aug. 2	Aug. 16
10 cm. plot	Height (cm.)	2.0	11.0	38.8	69	114	154	170	172	—
	Leaf dry weight (g.)	0.17	0.65	0.98	1.5	2.1	2.6	—	—	—
	Stem and petiole dry weight (g.)	0.08	0.32	0.92	2.1	3.6	4.7	—	—	—
	Root dry weight (g.)	0.23	0.35	0.6	1.0	1.2	1.2	—	—	—
	Stolon dry weight (g.)	0.07	0.14	0.3	0.6	0.8	0.8	—	—	—
	Total dry weight (g.)	0.55	1.46	2.80	5.2	7.7	9.3	—	—	—
20 cm. plot	Height (cm.)	2.0	6.4	16.0	44.0	65.0	101.0	154.8	190.3	209.7
	Leaf dry weight (g.)	0.17	0.7	1.4	2.9	4.7	6.4	7.9	9.2	9.8
	Stem and petiole dry weight (g.)	0.08	0.32	0.92	2.3	4.6	7.6	11.2	17.5	24.0
	Root dry weight (g.)	0.23	0.6	1.2	2.5	3.9	3.7	3.8	3.3	—
	Stolon dry weight (g.)	—	0.02	0.3	1.0	2.3	4.0	4.8	5.7	—
	Total dry weight (g.)	0.48	1.64	3.82	8.7	15.5	21.7	27.7	35.7	43.0
30 cm. plot	Height (cm.)	2.0	6.0	17.3	44.0	64.9	101.3	169.8	210.1	241.6
	Leaf dry weight (g.)	0.17	0.7	1.8	4.1	8.4	13.0	18.0	23.6	28.5
	Stem and petiole dry weight (g.)	0.08	0.32	0.92	2.5	6.8	12.5	21.5	37.0	56.0
	Root dry weight (g.)	0.23	0.61	1.2	3.3	5.2	6.0	6.9	7.5	14.9
	Stolon dry weight (g.)	—	0.01	0.3	1.2	2.3	5.2	7.9	10.3	16.1
	Total dry weight (g.)	0.48	1.64	4.22	10.1	22.7	37.0	53.3	78.4	115.5
100 cm. plot	Height (cm.)	2.0	6.0	15.8	29	51	75	108	161	210
	Leaf dry weight (g.)	0.17	0.70	1.6	3.6	8.7	19.5	36.0	58.0	86.0
	Stem and petiole dry weight (g.)	0.08	0.32	0.92	2.5	6.8	16.2	37.0	88.0	134.2
	Root dry weight (g.)	0.23	0.60	1.46	2.3	5.0	6.3	12.4	20.0	24.8
	Stolon dry weight (g.)	—	0.02	0.04	1.2	3.4	9.3	13.6	23.0	31.2
	Total dry weight (g.)	0.48	1.64	4.02	9.6	23.9	51.3	99.0	189.0	276.2



to 11.6 g. in the 20 cm. plot and 15.8 g. in the 30 cm. plot (a ratio of 1:1.9:2.8) was measured. On August 6, 1956 the weight of 6.5 g. in the 10 cm. plot, 33.8 g. in the 20 cm. plot and 72.4 g. in the 30 cm. plot (1:5.2:11.1) were measured.

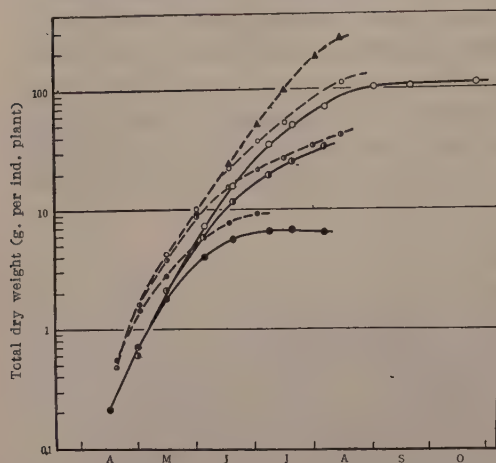


Fig. 2. Growth curve of total dry weight of entire plant: solid line and large mark: 1956; broken line and small mark: 1957;  $\Delta$ : spacing of 100 cm. See the note of Fig. 1.

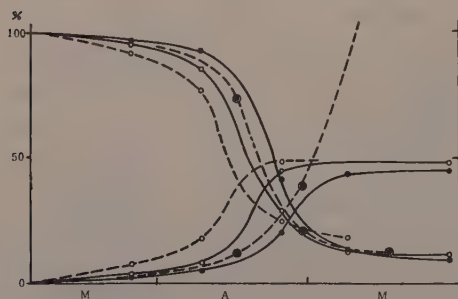


Fig. 3. Variation of dry weight of sprout and planted tuber with time: initial tuber dry weight: 100%;  $\bullet$ —: dry weight of tuber 3.42 g., and dark;  $\circ$ —: 1.05 g., dark;  $\circ$ —: 0.13 g., dark;  $\bullet$ —: 1.65 g., field.

over, light factor in a certain stratum of a community is decided by the total area of the leaves which are distributed above the stratum in question [14].

The time course of growth in leaf area of individual plant in each plot is illustrated in Fig. 4. The depressing effect of increasing density on the growth in leaf area is almost the same as in the case of the growth in leaf dry weight (Fig. 5). A reverse relationship between the total leaf area of an individual plant and its density is, of course, due to the increased competition for the total

area. The same phenomenon could be observed in the experiment of 1957, which is also shown in Fig. 2 and Table 1, too. On August 2, 1957 the mean dry weight of the sampled plants for the 10, 20, 30, and 100 cm. plots were 9.3, 35.7, 78.4, and 189.0 g. respectively (a ratio of 1:3.8:8.4:20.2).

In the earlier stage of development the relative growth rate, that means the ratio of the increase in dry weight per day to the total dry weight (the dry weight of planted tuber omitted), was as high as 0.06–0.075 in each plot. Then the ratio decreased rapidly with the progress of development in the highest density, and the value of less than 0.009 was found at the end of June. Contrary to this it decreased gradually in lower density plots (Table 2).

c. *Growth in leaves.* The leaf amount is naturally one of the most important factors for the photosynthesis. The substances produced excessively, that is, net production, are distributed to each organ in different proportions under the action of various environmental factors [6, 7].

The leaf area is an important item concerning the dry matter production in a community. More-

TABLE 2

The observed growth rate and net assimilation rate (NAR). Growth rate is expressed as the ratio of increase in dry weight  $\Delta W$  to total dry weight  $W$ . NAR is shown in  $\text{mg./cm}^2\text{./day}$

	10 cm. plot		20 cm. plot		30 cm. plot	
	Growth rate ( $\Delta W/W$ )	NAR ( $\text{mg./cm}^2\text{./day}$ )	Growth rate ( $\Delta W/W$ )	NAR ( $\text{mg./cm}^2\text{./day}$ )	Growth rate ( $\Delta W/W$ )	NAR ( $\text{mg./cm}^2\text{./day}$ )
April 14–April 30 (16 days)	0.070	0.80	0.059	0.60	0.059	0.60
April 30–May 15 (15 days)	0.059	0.74	0.075	0.70	0.075	0.72
May 15–June 4 (20 days)	0.037	0.50	0.047	0.49	0.049	0.53
June 4–June 19 (15 days)	0.022	0.43	0.043	0.46	0.069	0.75
June 19–July 8 (19 days)	0.009	0.12	0.027	0.25	0.066	0.61
July 8–July 20 (12 days)	0.003	0.04	0.021	0.20	0.032	0.49
July 20–Aug. 6 (17 days)	-0.003	-0.05	0.020	0.19	0.015	0.34
Aug. 6–Sept. 1 (25 days)	—	—	—	—	0.015	0.31
Sept. 1–Sept. 20 (20 days)	—	—	—	—	0.008	0.02

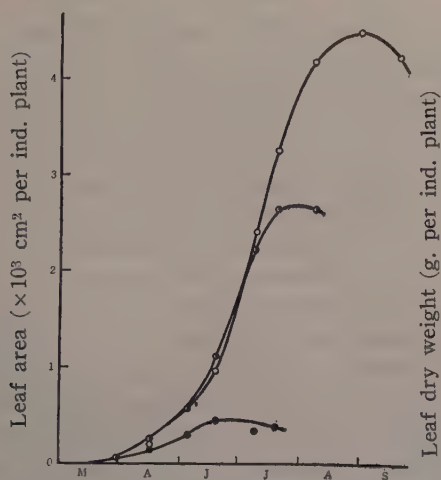


Fig. 4. Variation of leaf area of individual plant with time in 10, 20, and 30 cm. plot (1956). See the note of Fig. 1.

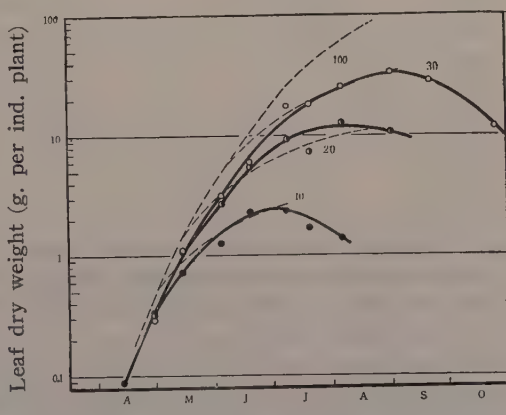


Fig. 5. Variation of leaf dry weight per plant with time and density in 1956, and 1957. See the note of Fig. 1.

amount of light received by the leaves of a plant in higher density plot. The similar phenomenon has been reported and discussed by many investigators using other plant species [4, 6].

The leaf dry weight at the successive stages of growth in each plot is shown in Fig. 5. It is evident that the growth in leaf dry weight declines significantly when the shading of leaves by the neighboring individuals begins, as the growth in the total dry weight of a plant does (Fig. 2). In the later stage, the leaf amount of individual plant in each plot showed a reverse relation to its density, and furthermore, the maximum leaf amount was attained sooner in a higher density than in a lower density. For instance, in the 1956 experiment the maximum value in the 10 cm. plot came to 2.3 g. dry weight (July 8), in the 20 cm. plot to 11.6 g. (Aug. 6), and in the 30 cm. plot to 31.9 g. (Aug. 31).

The ratio of the dry weight of leaves to that of entire plant which are given in Table 1, shows following facts: (1) In the early stage of development the most part (more than 75%) of stored and assimilated substance is utilized for the formation of new leaves (cf. Fig. 21). (2) Since then the formation of non-photosynthetic part, i.e., stems, roots, stolons, becomes relatively large. (3) After attaining the maximum value, the ratio decreases more rapidly in a higher density plot.

d. *Growth in stem.* Generally speaking, the growth in dry weight of stem including petioles shows the same tendency as those of the total dry weight and leaves of a plant (Fig. 2, 5, 6). Though the growth of a stem in

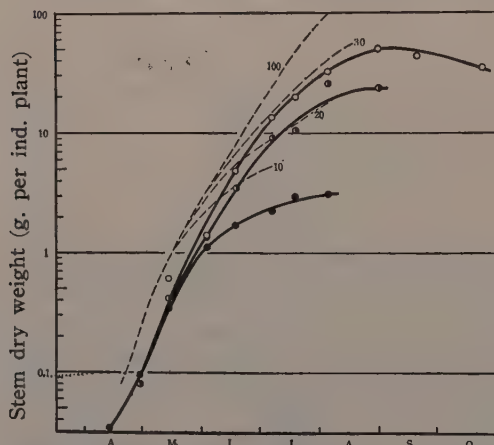


Fig. 6. Variation of stem and petiole dry weight of individual plant with time and density in 1956, and 1957. See the note of Fig. 1.

dry weight. It is also evident that shading of leaves makes the relative growth rate in stem larger than that in leaves.

e. *Growth in roots and stolons.* According to the ratio of the dry weight of aerial part to that of roots and stolons in Table 1, it is clear that in the early stage the most part of stored substances in planted tuber was used for

the growth of a stem in dry weight was in a reverse relation to the density, the effect of varying density on the growth of a stem was not so distinct as in leaves. On July 2, 1957 the leaf amount of 2.6 g. in the 10 cm. plot, 6.4 g. in the 20 cm. plot, 13 g. in the 30 cm. plot, and 19.5 g. in the 100 cm. plot (1:2.5:5.0:7.5) were measured, while the stem and petiole weight of 4.7, 7.6, 12.5, and 16.2 g. (1:1.6:2.6:3.4) was measured in respective plot.

Comparing the curves in Fig. 6 with these in Fig. 5, it is evident that the reduction of the growth rate in a stem originated in overcrowding appeared later than in the



the construction of the aerial part (cf. Fig. 3).

In the later stage, the growth in the subterranean part showed also in a reverse relation to the density (Fig. 7). In 1957, the maximum dry weight of 2 g. was measured in the 10 cm. plot, 9 g. in the 20 cm. plot, 31 g. in the 30 cm. plot, and 56 g. in the 100 cm. plot (1:4.5:15.5:28). This remarkable reduction of the growth in denser plot must be related closely to the depression of leaf amount caused mainly by the increase of shed leaves (cf. Table 5, Fig. 21).

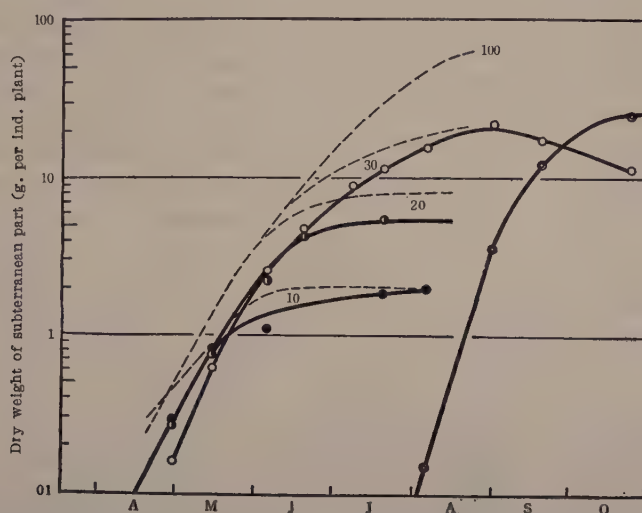


Fig. 7. Variation of dry weight of subterranean part (roots and stolons) with time and density.  $\odot$ : daughter tubers in the 30 cm. plot. See the note of Fig. 1.

The most of the subterranean part (90–95%) distributed in 0–10 cm. layer under the ground and the rest in the 10–30 cm. layer. According to the authors' observation, the formation of tubers began in August. In December of 1956 the average dry weight of tubers per individual in the 10, 20, and 30 cm. plots amounted to 4.1, 12.4, and 28.7 g., respectively. The increase of dry matter in the tubers in fall seems to be covered by the transported matter from other organs, especially from stem (cf. Table 1, Fig. 6, 21).

## 2. Growth of the community

It can be said roughly that the standing crop of a community indicates the integrated value of its dry matter production. Accordingly, the standing crop is useful for the approximate evaluation of dry matter production in a community. In the present study the standing crop per sq. meter was determined by the stratified clipping method [14] directly or by multiplying the mean values for plant weight (Table 1) by 100 for the 10 cm. plot, 25 for the 20 cm. plot, 11.1 for the 30 cm. plot, respectively.

a. *Growth in standing crop.* The time course of the growth of standing crop in each plot is shown in Fig. 8. The curves showed the exponential growth

in the earlier developmental stage, and then reduced gradually through the interaction between individuals.

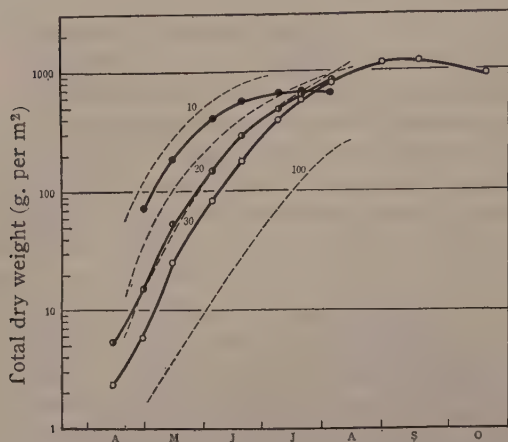


Fig. 8. Variation of standing crop (g. dry weight per 1 sq. meter) in 10, 20, and 30 cm. plot in 1956, and in 10, 20, 30, and 100 cm. in 1957 with time. See the note of Fig. 1.

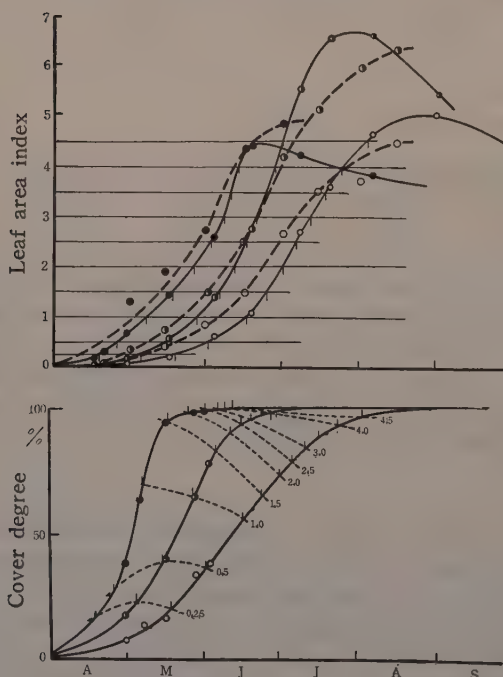


Fig. 9. Leaf area index (top) and percentage of cover degree (bottom) in 10, 20, and 30 cm. spacing communities at successive stage in 1956. Numbers in the bottom figure indicate the leaf area index. See the note of Fig. 1.

Therefore, the highest growth rate of the standing crop is observed at the lowest density. The difference in the standing crop in each plot was fairly large in the earlier developmental stage, but it reduced gradually with time. In other words the standing crop in dry weight seems to converge to some constant value, 1-1.2 kg/m<sup>2</sup>. [cf. 6, 10, 17]. It is also noticed that the denser the populations are, the sooner the maximum of standing crop will be observed.

#### b. Growth in leaf area

The depression of exponential growth of standing crop in a higher density will be largely due to the shortage of light intensity for the maximum production, owing to the shading of leaves in the overpopulated community. Under these circumstances the authors must deal with the time course of the leaf area growth, vertical distribution of leaves and the degree of shading of leaves in the community.

The total leaf area of the community per unit land area, that is leaf area index [cf. 14, 22], is shown in Fig. 9. In the earlier developmental stages the leaf area index increased exponentially, and the largest index is observed in the highest density, while the smallest in the lowest density. For the 1956 experiment, the index of 2.9 was measured on June 4 in the 10 cm. plot, 1.8 in the 20 cm. plot, and 0.6 in the 30 cm. plot. The results of the 1957 experiment showed the same ten-

dencies at all densities.

The maximum leaf area index of 4.5 was observed at the end of June in the highest density, while the corresponding indexes for the medium and the lowest density were 6.6 at the middle of July, and 5.0 at the end of August respectively. From the time course of leaf area curves in Fig. 8 and the facts mentioned above, it is clear that the maximum value is attained sooner in a higher density than in a lower density.

From the results of the observation and the theoretical analysis of the relationship between leaf area index and the relative light intensities in various communities Monsi and Saeki [14] made clear that the light intensity ( $I$ ) in a certain stratum in a community can be expressed by the following formula:

$$I = I_0 e^{-KF} \quad (1)$$

where  $I_0$  is the light intensity at the top of the community,  $F$  is the leaf area index from the top to the stratum in question, and  $K$  is the extinction coefficient, which is larger in a plant community with larger leaves than that with smaller leaves. They also demonstrated that the number of leaf area index in broad-leaved herb communities falls in the range of 4–6. The same number of index has been reported in woody communities by many investigators [e.g. 12, 16].

The changes of a productive structure and the relative light intensity in the community with time are illustrated in Fig. 10. The maximum leaf area index of the communities of *Helianthus tuberosus* with various densities is 4–6 as already mentioned. In Fig. 10 it is evident that the relative light intensity under foliage varies widely with the developmen-

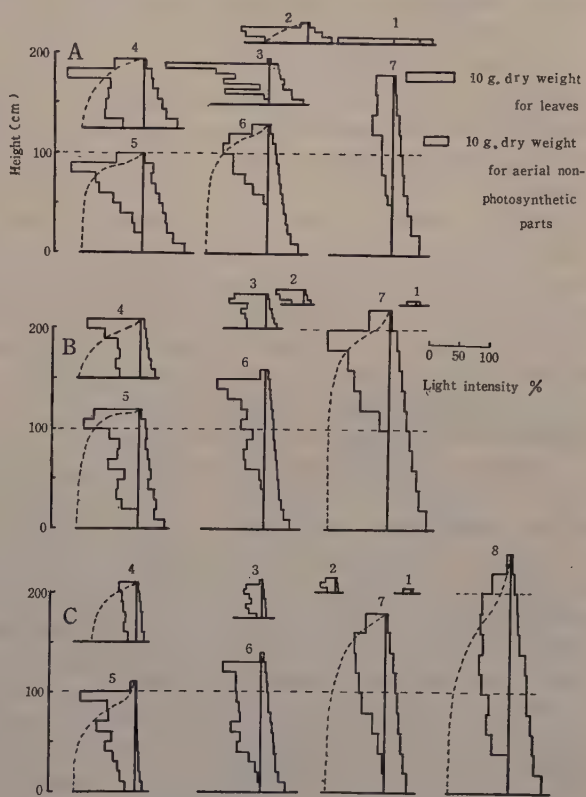


Fig. 10. Development of productive structure and relative light intensity in the communities with different spacing at successive stages in 1956. Dry weights of photosynthetic part in each 20 cm. layer, per  $50 \times 50$  cm<sup>2</sup>. land area are shown in the left side of longitudinal line, and non-photosynthetic part in the right side. Broken line shows relative light intensity.

A: 10 cm. plot; B: 20 cm. plot; C: 30 cm. plot.

1: April 30; 2: May 17; 3: June 4; 4: June 19; 5: July 8; 6: July 20; 7: Aug. 6; 8: Sept. 1.



tal stage and the thickness of foliage or number of leaf area index.

Using the leaf area index and the relative light intensity in Fig. 9 and 10, the authors can calculate the constant  $K$  by Equation (1). The value of  $K$  frequently fluctuated to some extent, but the mean value of  $K$  was ca. 0.9. The value of  $K$  varies mainly owing to the arrangement, direction and light transmissibility of leaves [9, 14]. It is sure that the arrangement and direction of leaves in a community relate closely to shading degree of leaves.

The importance of mutual or self shading on the growth or the dry matter production of a community has been recognized by several investigators. For *Chlorella* population Tamiya et al. [18] pointed out the remarkable influence of mutual shading upon the light intensity in the population and discussed its effect upon the photosynthesis.

Recently Hodgson et al. [5] dealt with the effect of self-shading in the artificial population of *Vicia faba* upon the growth of individual and each organ. In their report, however, the degree of self-shading in the population was not determined directly, but expressed by the decrease of light intensity under the foliage.

In the authors' experiment the degree of shading is determined by the following principles. If there exists no shading within a foliage, the cover degree must be 100% when the leaf area index is one. That is, however, contrary to the facts, and the percentage of cover degree is always smaller than 100%. It is obvious that the difference between them shows the degree of shading in leaves. In the authors' experiment, therefore, the determination of cover degree in various developmental stages is necessary to know the degree of shading besides the determination of leaf area index.

The cover degree in a community was determined by a photographic method. The results are shown in Fig. 9 together with the results of measuring leaf area index. The cover degree was small in the earlier stage, especially in the lower density plot. When the leaf area index got to one, the cover degree of 68% was determined in the highest density, while 65% in the medium density and 56% in the lowest one. In the developmental stage having leaf area index 2, the cover degree of 98% was measured in the 10 cm. plot, 90% in the 20 cm. plot, and 73% in the 30 cm. plot.

As shown in Fig. 9, the cover degree increased more rapidly in denser plot, and the cover degree of ca. 100% was attained at the beginning of July in the highest density plot, at the beginning of August in the medium, and at the end of August in the lowest ones, while the number of leaf area index at the corresponding stages was 2.5, 5.0, and 5.0 respectively. It is very remarkable that the amount of shed leaves increased simultaneously with attaining 100% of cover degree (cf. Table 5).

The said shading degree must affect the dry matter production of a plant community, and it is highly desirable in near future to obtain more accurate information concerning this problem, because the condition of penetration of direct sunshine has hardly been considered in the calculation of dry matter production.

### 3. Analysis of community growth in different densities

It is needless to say that community growth is equal to the sum total of the growth in individuals which constitute the community. The growth of an individual under the condition of different densities is reduced more or less by the competition among the neighboring individuals for necessary factors. This process has been called "density effect".

For the causes of the density effect Clements et al. [4] pointed out the competition of roots for water and nutrient salts firstly and then the competition of leaves for solar radiation, according to the results of their experiments on the artificial community of *Helianthus annuus*.

In the present experiment the variation of soil moisture with time inside and outside the community was measured. The data on August 15 and 16, 1956 are shown in Fig. 11. For about two weeks before that period the weather was fine and there was no rain, except for the shower in the middle of night of the former day. As the quantity of the precipitation was small, the soil moisture content in the surface layer in the community showed no significant increase, while the rapid increase was recognized in the bare land (cf. Fig. 11). From the facts mentioned above, it may be said that it was the driest period in the course of the present experiment.

The daily fluctuation of soil moisture in surface layer was larger in the open owing to the vigorous evaporation from the soil surface in the heat of the day than in the community, and decreased in a higher density plot. The minimum content of soil moisture in the community was 23% at the surface of soil and 27% at the 10 cm. depth, while the wilting coefficient of the soil was 23% (15 Atm.). Furthermore, an active stomatal transpiration (0.2–0.4 mg.  $H_2O/cm^2/min.$ ) was determined with a torsion balance in midday without any symptom of wilting (Fig. 12). From these and other unpublished data the authors concluded that the water did not play upon the growth of the community as a limiting factor.

The content of nutrient salts such as nitrogen, phosphorus, potassium, calcium, magnesium and iron, differed widely with the age of leaves and stems

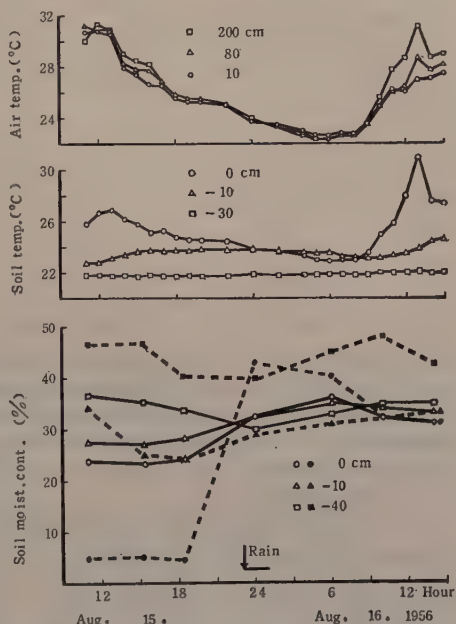


Fig. 11. Daily fluctuation of air and soil temperature and soil moisture content in the 20 cm. plot on Aug. 15–16, 1956. It rained in the middle of night in very small quantity. Broken lines: soil moisture content in bare land.

(Fig. 13). It is clear that nitrogen, phosphorus and potassium are mobile in a plant body, and the accumulation of these elements is large in the young and active leaves and stems. On the contrary, calcium, magnesium and iron are immobile, and they are found in the old and inactive ones in a large quantity [20, 21, 23]. Multiplying the concentration of these elements by the standing crop, we can obtain the total amount of elements accumulated in the community.

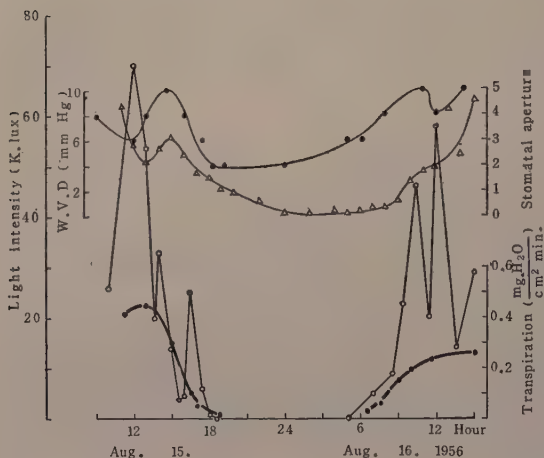


Fig. 12. Daily fluctuation of transpiration, stomatal aperture, water vapour deficit and light intensity on Aug. 15-16, 1956. —●—: transpiration (mg.  $H_2O$  / $cm^2$ ./min.) —△—: stomatal aperture measured by infiltration method; —●—: water vapour deficit; —○—: light intensity in lux.

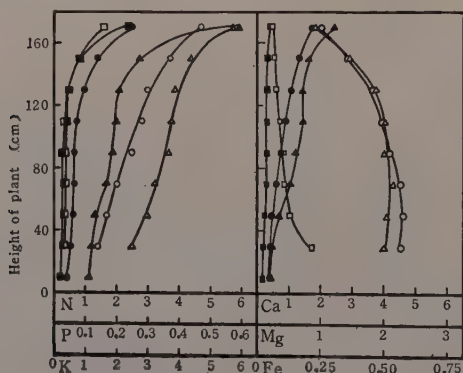


Fig. 13. Concentration of nutrient salts in leaves and stem in 30 cm. plot (1956). Concentration is shown in mg. per 1g. dry weight. ○: N and Ca in leaves; ●: N and Ca in stem; □: P and Mg in leaves; ■: P and Mg in stem; △: K and Fe in leaves; ▲: K and Fe in stem.

By the maximum growth, the community with the 10 cm. spacing absorbed about 11.0 g. of N, 0.9 g. of P, 7.8 g. of K, 11.7 g. of Ca, 9.0 g. of Mg, and 0.2 g. of Fe per sq. m. (July 8); that of the 20 cm. spacing, 18.3 g. of N, 2.0 g. of P, 10.5 g. of K, 13.3 g. of Ca, 9.0 g. of Mg, and 0.9 g. of Fe (Aug. 6); and that of the 30 cm. spacing, 17.0 g. of N, 2.7 g. of P, 8.0 g. of K, 17.4 g. of Ca, 10.5 g. of Mg, and 0.6 g. of Fe (Aug. 31).

Apart from the chemical analyses of plant ashes, the analyses of soil extract were carried out (Table 3). The concentration of the available nutrient salts in the soil extract showed no significant difference between the sample taken before the planting and that sampled at the stage of the maximum growth, in spite of such vigorous absorption of salts as mentioned above. Even in the later stage, the authors could not recognize any visible symptom of deficit in the nutrient elements; such as chlorosis by nitrogen deficiency, dark greening of leaves by phosphorus deficiency, and necrosis of apical bud by calcium deficiency [cf. 20, 21].

Based on these facts we may also conclude that the nutrient salts absorbed did not limit the growth of community



TABLE 3

Chemical analyses of soil extracts. Concentrations of each element are shown in p.p.m., and total N in percent.

	April 30		June 4		August 6	
	Bare land	30 cm. plot	Bare land	30 cm. plot	Bare land	30 cm. plot
Total N	—	0.42	—	0.42	—	0.42
NH <sub>3</sub> -N	24.1	17.1	25.5	25.5	20.4	25.9
NO <sub>3</sub> -N	17.8	15.5	6.0	8.2	16.7	8.8
K	100	122	116	77	139	68
Ca	1524	1244	—	—	1422	1636
PO <sub>4</sub> -P	1.4	1.0	1.8	1.5	0.9	1.0
Fe	7.5	7.5	—	—	6.5	7.5

principally. Hereupon, we must deal with the effects of the light factor upon the growth of the communities with different densities. As the growth rate in dry weight is determined by the balance-sheet of photosynthetic and respiratory processes during a certain period of time, it is sure that the light factor, which determines the CO<sub>2</sub>-assimilation rate directly, plays an important role for the growth of the community.

For the purpose of analyzing the growth of a community, net assimilation rate (NAR) has often been adopted by many investigators [cf. 1, 6, 22 etc.]. In general, NAR is represented by the following equation:

$$\text{NAR} = \frac{1}{F} \times \frac{dW}{dt} \quad (2)$$

where  $F$  is the total leaf area or the leaf weight of the plant and  $dW$  the increase in dry weight of the standing crop in a period of  $dt$ . In this case, the amount of shed part during a period of  $dt$  must be taken into consideration [cf. 11].

In the present experiment the time trends of NAR (mg./cm<sup>2</sup>./day) are shown in Table 2. NAR was large in the earlier stage of development in every plot, and the higher the density of the community, the sooner the maximum of NAR was attained. Then NAR decreased with time, and the higher the density, the more rapidly the decrease of NAR occurred.

The growth of a plant is generally formulated as following [2]:

$$W = W_0 e^{\lambda t} \quad (3)$$

where  $W_0$  is the initial dry weight of the total plant and  $W$  the dry weight after the period of  $t$ .  $\lambda$  is a growth rate, and it varies with developmental stages. Accordingly, the time trends of the growth curve are decided by the variation of magnitude of  $\lambda$  with time.

Growth rate,  $\lambda$ , is expressed by a ratio of the increase in dry weight  $\Delta W$  in a period  $t$ , to the total dry weight of the plant  $W$ . Therefore,  $\lambda$  can be ex-

pressed in the following equation:

$$\lambda = \frac{\Delta W}{W} = \frac{F \cdot a - F \cdot r - C \cdot r_c - D}{F + C} \quad (4)$$

where  $F$  and  $C$  represents the total dry weight of the photosynthetic and non-photosynthetic organs, respectively,  $a$  and  $r$  the rates of assimilation and respiration per unit dry weight of the former,  $r_c$  the respiration rate per unit dry weight of the latter, and  $D$  the dry weight of shed part in the period [cf. 6, 11]. In Iwaki's experiment [6] the amount of shed leaves was out of the question because of the character of his experimental material, but in the present study it could not be disregarded.

To elucidate the action of the environmental factors on the growth of plants, it is necessary to make clear the relation between each of the physiological processes which determine the magnitude of  $\lambda$ , and environmental factors quantitatively. In addition to this, it is evident that the distribution rate of net production to the photosynthetic and non-photosynthetic organs is influenced markedly by the environmental factors, and changes the development of productive structure of the community. For this reason the assessment of the distribution rate under the given condition is necessary for the growth analysis of plant community.

a. *Assimilation (a)*. It is difficult to elucidate the mean daily assimilation per unit amount of leaves under the field conditions directly. In the present

study the amount of assimilation was calculated indirectly, combining the light assimilation curve of a leaf with the daily curve of light intensity outside and inside the community [cf. 6, 13].

In the present experiment there was significant difference among the features of assimilation curves of different aged leaves [19] (Fig. 14). The light assimilation curve of a young leaf was rather similar to that of an old leaf except for a large respiration rate and a little high compensation point, while that of a matured leaf was of a typical sun leaf type. The assimilation rate of a matured leaf in the field under

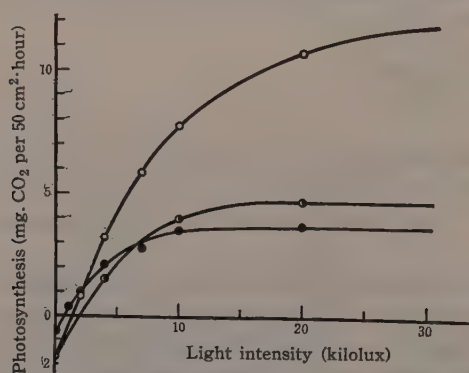


Fig. 14. Light-photosynthesis curves of young, matured, and old leaf. Photosynthesis is expressed in mg. CO<sub>2</sub> per 50cm<sup>2</sup>. per hour, and light intensity in kilo-lux. ○: young leaf; ○: matured leaf; ●: old leaf.

full light condition of midday showed a good fitness with corresponding value in the laboratory experiment. Therefore, it may be said that the assimilation curves constructed from the measurements in the laboratory provide us appropriate values expected under field conditions. When a leaf develops, it stays only 5-6 days in the young leaf type and then proceeds to the matured leaf type.

Therefore, the distribution of young leaves is limited to the top of plant, and the ratio of them to the total amount of leaves is very small, especially in the later developmental stage. The old leaf type is recognized in the leaves which are distributed under the leaf canopy in a small quantity, and illuminated by weak light intensity. From this reason it was assumed that all leaves belonged to the matured leaf type, in order to make the calculation easier.

As regards the mean light intensity in the open, the intensities of 50 Klux and 10 Klux were employed for fine and cloudy conditions [13]. For elucidation of the daily course of solar radiation in the stand, it is necessary to estimate the duration of bright sunshine in per cent (Fig. 15). The time in the morning and evening when the illumination was below the compensation point (ca. 1400 lux) was omitted from the assimilation day length. Furthermore, the latter was divided into two parts, i.e. hours when the plants received direct sunshine and hours when they received only diffused light, according to the duration of bright sunshine in per cent. The light intensity in the community was calculated by the relation of light intensity and leaf amount shown in Fig. 10.

The daily assimilation of the community is given by summing the corresponding quantity in each stratum which construct the community.

b. *Respiration of photosynthetic and non-photosynthetic organs ( $r$  and  $r_c$ )*

The respiration rate of leaves shows a marked variation with the leaf age (Figs. 15 and 16), but not with the density [cf. 6]. The leaves of *Helianthus tuberosus* develop opposite on a stem from the first node to the nineteenth or twentieth node thereabout, but alternatively above the latter node, without regard to the density. The leaf age therefore is expressed by the number of the node on which a pair of leaves situate, and in the latter case two neighboring alternate leaves are counted as a pair of opposite ones.

As an example, the respiration rates of leaves in the 30 cm. plot measured under the condition of 20°C are shown in Fig. 16. The respiration rate was always large in the apical young leaf, and reduced with the increase of leaf age. It is quite clear that the maximum respiration rate in the apical leaf decreases

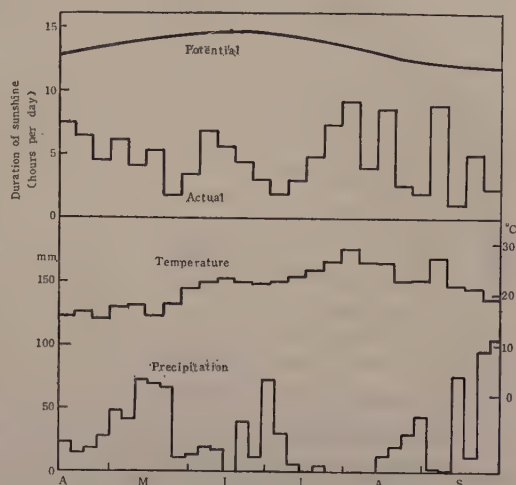


Fig. 15. Fluctuation of duration of sunshine, air temperature, and precipitation in 1956. Actual duration of sunshine is shown in the mean value for a week (hours per day); temperature in the mean value for a week; precipitation in the mean value for 5 days (mm./day). (After Tokyo District Meteorological Observatory).



gradually with the march of the developmental stage. For instance, the maximum rate of 8.5 mg.  $\text{CO}_2/\text{g.}/\text{hr.}$  was measured on May 17, 6 mg. on July 27, and 3.5 mg. on September 22. It is also worth noticing that the decrease of the respiration rate in the matured and old leaves seems to converge to some constant value, i.e. 0.7–1.0 mg.  $\text{CO}_2/\text{g.}/\text{hr.}$  The same phenomena were observed in the leaves of stands in the other spacing plot, and it may be expected that these will be observed widely in other species.

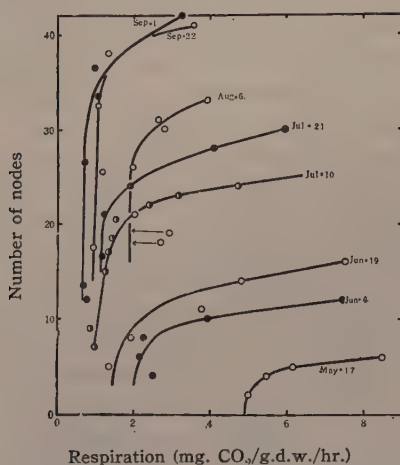


Fig. 16. Variation of respiration activity of leaves at 20°C with leaf age and with time in 1956. Respiration: mg.  $\text{CO}_2$  per 1 g. dry weight per hour. Number of nodes indicate leaf ages. See text.

The total amount of respiration is necessary to the calculation of growth rate. The respiration amount of leaves was obtained multiplying the respiration rate of different aged leaves by their weight. For instance, the value obtained in the 30 cm. plot at 20°C is given in Fig. 17, together with their dry weight.

For the sake of assessment of the respiration amount under the varying field condition, some micro-climatic observations on the diurnal course of temperature in the community were performed (cf. Fig. 11). The daily variation of the air temperature and that of the soil temperature at 0–10 cm. depth in the communities were very conditional, but the mean values did not differ markedly from those of the open place except the temperature near the ground surface.

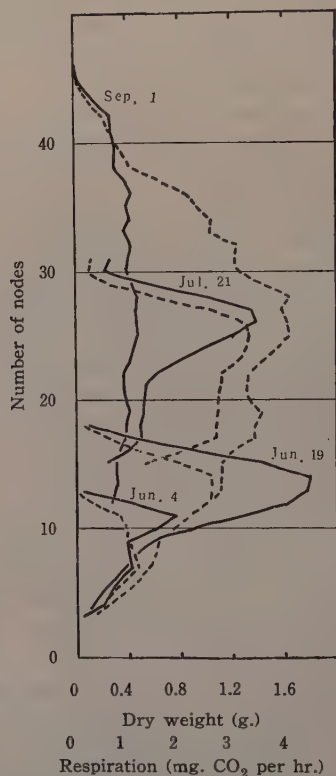


Fig. 17. Vertical distribution of leaf size and respiration loss in leaves at 20°C (1956). Broken line: leaf size in g. dry weight per pair of leaves; solid line: respiration loss in mg.  $\text{CO}_2$  per pair of leaves per hour.

As the result of this, the macro-climatic temperature data of the Tokyo District Meteorological Observatory were adopted here for the simplicity of calculation (Fig. 15).

Though the respiration of leaves varied markedly with leaf age, the mean values of respiration in leaves of a plant in each density were similar to each other, and decreased with development. The variations of the mean values of respiration in leaves, stems and subterranean part with time are shown in Fig. 18. Combining the respiration rate at 20°C (Fig. 18) with the dry weight of leaves (Fig. 10, Table 1), temperature change (Fig. 15) and  $Q_{10}$  of respiration which was determined as 2.0 in a temperature range of 9°–28°C (Table 4), the respiration amount of the foliage under the field condition was obtained (Fig. 19).

The respiration amount of petioles and stems or subterranean organs in the stand was, after measurement of respiration rate at 20°C, calculated by the same way as in the case of leaves mentioned above (cf. Table 4, Figs. 18, 19).

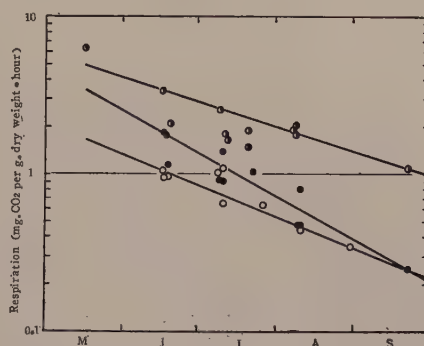


Fig. 18. Variation of respiration activity of leaves, stem, and subterranean part at 20°C with time. ○: respiration activity of leaves; ●: respiration activity of stems; ○: respiration activity of subterranean part.

TABLE 4

Temperature coefficients of respiration. Temperature range 9–28°C

Organ	Date	Range °C	$Q_{10}$
Leaf	May 7	16–26	2.0
Stem	Sept. 23	9–22	2.2
Root	Sept. 3	13–28	2.3
Whole plant	May 7	16–26	1.8

In Fig. 19 the respiration amounts of foliage, stems, and subterranean parts are shown in g. dry weight per day. In the highest density the total amount of respiration loss per day increased markedly in June, but in the medium and the lowest density in August in company with the maximum increase in dry weight (cf. Table 1, Figs. 8, 12).

c. *Net assimilation of leaves ( $F(a-r)$ )*. In the young leaves distributed in the top of the community, the net assimilation should be small and occasionally be negative, because of low assimilation and high respiration activity accompanied with high physiological activities such as growth, salts accumulation etc. (cf. Fig. 13). The rapid growth in apical part seems to be supported by large net assimilation of matured leaves, though the direct evidence is not yet obtained.

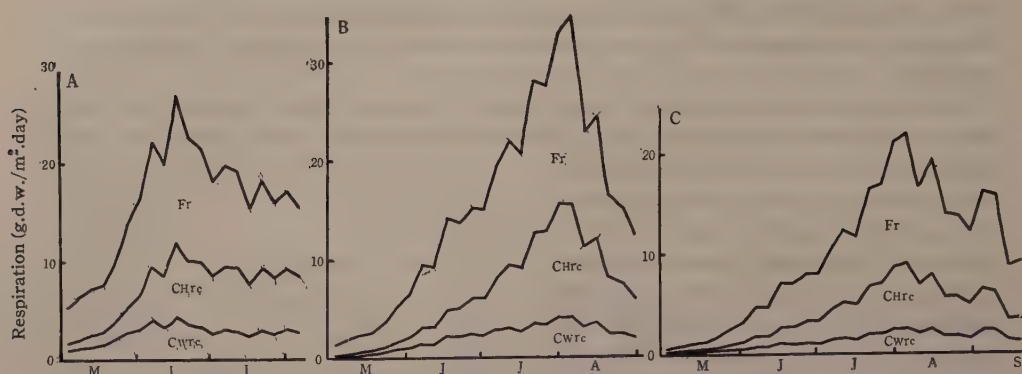


Fig. 19. Variation of calculated daily respiration loss in leaves, stems, roots, and stolons with time in 10 (A), 20 (B), and 30 cm. (C) plots under field conditions. Respiration: g. dry weight per sq. m. per day; Fr: respiration in leaves; CHrc: respiration in stems; CWrc: respiration in roots and stolons.

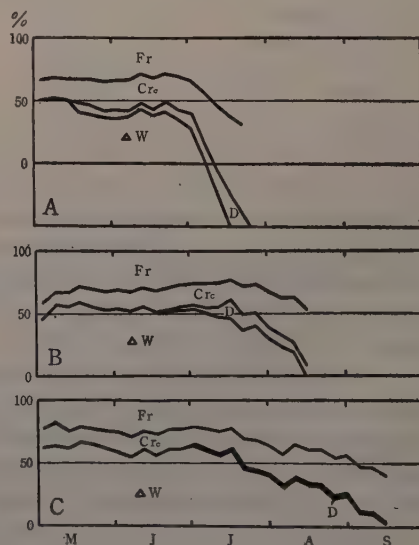
From the facts stated before, also the net assimilation in old leaves seems to be small. It is almost sure that the most part of the net assimilation of the foliage is owing to the mature leaves of the high activity.

The amount and proportion of leaves which are influenced markedly by the density are very important for the development of the community. The leaves situated in the illumination below the compensation point are sooner or later shed without gaining any supply of substance from other leaves (Kimura, unpublished). This phenomenon may also play an important role in the modification of community structure by reducing the old leaves in the course of development (cf. Fig. 10).

d. *Distribution of the assimilated substance to each organ.* In community growth the net production is used to construct the new structure under the influence of various environmental factors. Accordingly, we can not analyze the growth of a community dynamically without the knowledge of the relation between the distribution of the net production and the environmental factors. To obtain some information on this problem, the distribution of photosynthesized substance to each organ was pursued in each plot.

The results obtained are illustrated in

Fig. 20. Distribution of gross production to increment in dry matter, and respiration of photosynthetic and non-photosynthetic parts. Difference between dry matter increase and dry weight of shed leaves (D) means net increase ( $\Delta W$ ). See the note of Fig. 19.





Figs. 20 and 21. The percentage of the gross production distributed into increment in dry weight of total plants and that consumed by respiration of photosynthetic and non-photosynthetic organs are illustrated in Fig. 20 (cf. Figs. 8, 19, Table 1). So far as the quantity concerned, a part of this increment was taken away by the amount of shed leaves in concerned period, and the net increase was given as a difference between them. It is very interesting that percentage of each fraction is independent on the densities of the communities excepting the one in later stage of development, and about 50% of the gross production was used for the increment in dry weight and the rest respired throughout the growing season, though there was some increase in percentage of substance used for the increment in lower density. In later stage the most part of photosynthesized substance was immediately respired by the leaves, and this brought about the rapid increase of percentage in Fr. It is also evident that in later stage of development the denser the communities are, the sooner the increase of respired substance or the decrease of increment in dry weight appears.

Fig. 21 shows the time course of the dry weight of distributed substance to increment and respiration of leaves, stems, roots, stolons and daughter tubers, taking the amount of shed leaves into consideration (cf. Figs. 5-7, 19, Table 1,

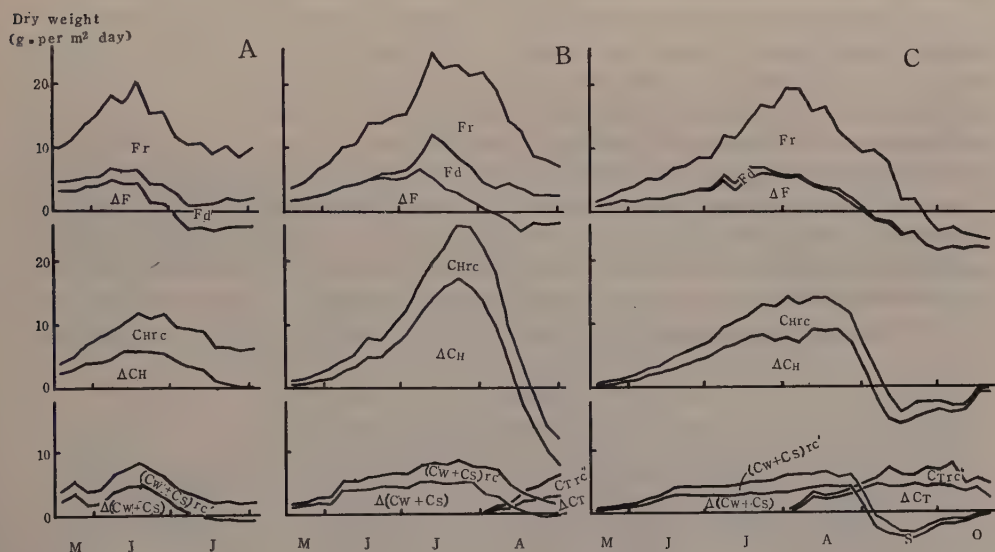


Fig. 21. Variation of distributing amount (g. dry weight per sq. m. per day) of gross production to increment in dry weight and respiration of each organ, and amount of shed leaves (g. dry weight per sq. m. per day) in 10 (A), 20 (B), and 30 cm. (C) plots with time (1956). F: leaves; Fd: shed leaves; Fr: respiration of leaves;  $\Delta F$ : dry matter increase in leaves; CH: stems; CHrc: respiration of stems;  $\Delta CH$ : dry matter increase in stem; CW: roots; CWrc': respiration of roots;  $\Delta CW$ : dry matter increase in roots; CS: stolons; CSrc': respiration of stolons;  $\Delta CS$ : dry matter increase in stolons; CT: daughter tubers; CTrc': respiration of tubers;  $\Delta CT$ : dry matter increase in tubers. See Fig. 20.

5). The distributed amount and the ratio of dry weight increment to respiration in dry weight, changed with organ and time. In the later stage, as the loss of dry matter in leaves and stems by respiration, translocation and shedding was not covered by the distributed substance, the balance-sheet of gained and lost substance became negative. It is worthy to notice here that the decrease of growth in the denser community may be caused by the increase of shed leaves or the decrease of active leaves.

e. *Theoretical construction of the growth curve.* The theoretical construction of the growth curve of a community based on the growth analysis is very few. Recently Iwaki carried out the calculation of the growth curve of buckwheat community using the results of measurement of some physiological activities and environmental factors, and he showed the usefulness of his procedure for the growth analysis [6]. The authors also tried the construction of the growth curves of *Helianthus tuberosus* communities with different densities for the purpose of testing the fitness of our growth analysis.

The procedure for calculating community growth employed in the present work seems to be similar to that of Iwaki's report, but differs, in some points. In this study the calculation of the growth was undertaken using the Equation (3) mentioned above, in order to taken up the growth as successive as possible.

For the application of Equation (3), it is needed firstly to know the magnitude of growth rate  $\lambda$ , which is expressed in Equation (4), in every developmental stage. It is worthy to summarize here the procedure to obtain the value of growth rate. 1) Combining the relative light intensity in the communities (Fig. 10), with the daily course of absolute light intensity in open (Fig. 15), the vertical distribution of absolute light intensity in the communities was obtained. 2) The daily photosynthesis, that is gross production, was calculated using the light intensity at each stratum, the total leaf area in each stratum, and the light-real photosynthesis curve obtained from that of Fig. 14. 3) The daily amount of respiration loss in each organ under natural temperature condition (Fig. 15) was determined by converting the respiration at 20 or 25°C (Fig. 16, 17) by the

TABLE 5  
The amount of shed leaves in dry weight in 1956

	10 cm. plot		20 cm. plot		30 cm. plot	
	g./ind.	g./m <sup>2</sup> .	g./ind.	g./m <sup>2</sup> .	g./ind.	g./m <sup>2</sup> .
-June 4	0.289	28.9	0.096	2.4	0.096	1.07
June 4-June 19	0.576	57.6	0.192	4.8	0.192	2.13
June 19-July 8	1.15	115	1.065	26.4	0.869	9.60
July 8-July 20	1.54	159	4.22	105.5	2.21	24.55
July 20-Aug. 1	2.11	211	—	—	—	—
Aug. 1-Aug. 7	—	—	7.58	189.5	2.66	29.55
Aug. 7-Sept. 1	—	—	9.98	250	3.71	41.2
Sept. 1-Sept. 22	—	—	—	—	4.29	47.7

application of temperature coefficient of respiration activity measured (Table 4). By means of assimilation and respiration value of each organ per day obtained by the foregoing procedure, the growth rate  $\lambda$  per day can be calculated. As the mean values of solar radiation and temperature for seven days are employed in present procedure,  $t$  in Equation (3) is seven. Then we can obtain the dry weight of plants after seven days following Equation (3).

The increase in dry weight, that is net production, is utilized for the growth of photosynthetic and non-photosynthetic part in different proportions under the influence of environmental factors. 4) As for the distributing ratio of the net production to each organ the values which are given from the observed data (cf. Table 1, Fig. 21) were employed. Multiplying the net production by these ratios, the dry matter increment in each organ for a week was determined. 5) The increase of dry matter in leaf amount was converted to that of leaf area, and then the leaf area index of the community after a week was obtained. The light intensity in the plant community with newly added leaves was determined by means of the leaf area index and the extinction coefficient  $K$  ( $=0.9$ ) mentioned above. In this case it was assumed that the leaves situated in the illumination below the compensation point (1400 lux) were shed.

Repeating these procedures to each plot, the rational growth curves of the communities of *Helianthus tuberosus* with different densities were made.

The calculation of community growth was carried out based on the data from April 30, 1956 to July 15, 1956. The growth rate  $\lambda$  in each plot calculated by foregoing procedures at successive stages is shown in Table 6. Comparing the calculated growth rate in Table 6 with observed one in Table 2, we can find considerable agreement among them, though there exists some differences between them in the later developmental stages.

The growth curves constructed theoretically by the procedure mentioned above are shown in Fig. 22 with the curves observed (cf. Fig. 8). The theoret-

TABLE 6

The calculated growth rate. Expressed as the ratio of increment in dry weight to the total dry weight (Calculating procedure in the text)

Date	10 cm. plot	20 cm. plot	30 cm. plot
May 1-May 7	0.074	0.066	0.082
May 8-May 14	0.049	0.071	0.068
May 15-May 21	0.046	0.083	0.070
May 22-May 28	0.025	0.069	0.063
May 29-June 3	0.020	0.055	0.059
June 4-June 10	0.028	0.054	0.057
June 11-June 17	0.018	0.038	0.050
June 18-June 24	0.016	0.023	0.046
June 25-July 1	0.010	0.020	0.040
July 2-July 8	0.009	0.031	0.037
July 9-July 15	—	0.036	0.034



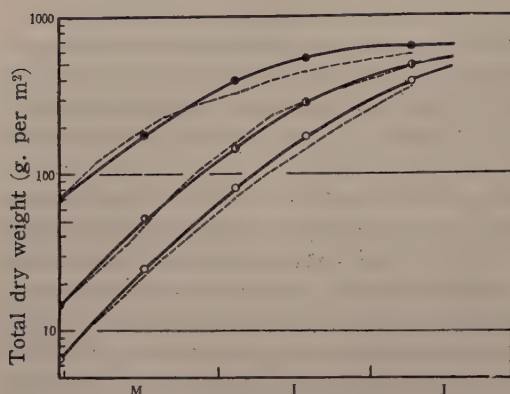


Fig. 22. Observed and calculated growth curves of the communities with different spacing of 10, 20, and 30 cm. Solid line: observed; broken line: calculated. See the note of Fig. 1.

tical curves show the same tendencies in each density as in the curves observed, though the absolute values differ each other to some extent, especially in the later stage of development.

From these facts mentioned above, it may be concluded that the authors can succeed to construct the approximate growth curve of the plant community theoretically under given field condition, if some environmental factors and physiological properties which influence upon the growth of plant community are given previously.

## SUMMARY

For the purpose of analyzing the growth process of plant community under varying environmental conditions with time and density from view point of dry matter production, the growth analytical study on artificial communities of *Helianthus tuberosus* with a spacing of 10, 20, and 30 cm. in 1956, and a spacing of 10, 20, 30, and 100 cm. in 1957, was carried out. Primarily the growth of individual plant in every density was studied to obtain fundamental data needed for the analysis of the growth of communities. Then the time courses of the growth of communities were pursued on the basis of the study mentioned above, and furthermore, theoretical analysis of them was carried out. The main results obtained are as follows.

### I. Growth of individual plant

(1) At the early stages, plant height (Fig. 1), leaf area (Fig. 4), and dry weight of each organ (Figs. 5-7) and entire plant (Fig. 2) did not show any significant difference in each density, but in later stages, some remarkable differences were observed.

(2) The most part of aerial and subterranean part formed in the early stage was made from the stored substance in planted tuber, and the photosynthesis in leaves did not play any important role in this stage. The economic ratio in conversion from reserved substance into active organs was ca. 0.5 (Table 1, Fig. 3).

(3) At the later stages, the growth reduction in height, leaf area, and dry weight occurred primarily in the plant of higher density, and it removed successively in the plant of lower density with time. Therefore, the denser the plots were, the sooner the maximum of growth in each organ and entire plant was

attained (Figs. 1-2, 4-7). To make clear the causes of these reductions in growth the meaning of shading of leaves was discussed.

(4) The maximum growth of a plant in each plot showed a reverse relation to the density. The reducing effect of overcrowding appeared more markedly in the growth of leaves and subterranean part than in that of stem.

(5) The relative growth rate in dry weight of entire plant per day was as high as 0.06-0.075 in the early stage, and it decreased rapidly with time, especially in denser plot (Table 2).

## II. *Growth of the community*

(6) At the early stages, the largest standing crop was observed in the highest density, and the smallest one in the lowest. The difference between them, however, reduced gradually with time. In the later stages, the standing crop seemed to converge to some constant value independently of the density (Fig. 8).

(7) According to the measurements of soil moisture, transpiration and concentration of nutrient salts in soil extracts and plant ashes (Fig. 11-13, Table 3), it was made clear that the water and nutrient factors did not limit directly the growth of standing crop even in the highest density.

(8) The development of productive structure and the relative light intensity in the community were pursued in every density. The maximum leaf area index was found in the range of 4.5-6.6, and extinction coefficient was ca. 0.9. The relation between leaf area index and cover degree was studied. After the cover degree of ca. 100% was attained, the amount of shed leaves increased rapidly (Fig. 9, Table 5).

(9) As the assimilation activity of leaves varied widely with leaf age, three types of light assimilation curves were distinguished (Fig. 14). Combining the curve of matured leaves with leaf amount and light factor, the gross production of the community needed for the calculation of growth rate, was obtained.

(10) The respiratory activity at 20°C was high in apical and young leaves, and it reduced to a constant value in matured and old ones (Fig. 16). The respiratory activity of other organs at 20°C was also measured frequently.  $Q_{10}$  of respiration was ca. 2.0 (Table 4). Using the data of respiration states (Figs. 17-18), dry weight of each organ (Figs. 10, 17),  $Q_{10}$ , and temperature in the field (Fig. 15), daily respiration loss of the community was estimated (Fig. 19).

(11) It was made clear that in the growing season ca. 50% of gross production was utilized for the increment of dry matter, and the rest was respired, though some increase of the former in lower density was recognized (Fig. 20). The distributed amount of gross production to each organ was studied at successive stages, considering the amount of respiration and of shed leaves (Table 5, Fig. 21).

(12) Using above mentioned values the growth rate  $\lambda$  was calculated by Equation (4). The calculated values showed considerable agreement with those observed (Table 2, 6).

(13) According to Equation (3) the dry weight of the communities after certain period was calculated theoretically. Repeating this procedure, the growth

curves of the communities were constructed. The theoretical curves showed considerable agreement with those observed (Fig. 22).

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# AN ECOLOGICAL ANALYSIS OF THE DISTRIBUTION OF BROAD-LEAVED EVERGREEN TREES, BASED ON THE DRY MATTER PRODUCTION

by

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## I. INTRODUCTION

The existence of a plant community in a certain region is due to the fact that the plant species which constitute the community are growing and distributing themselves in the region. The structure and composition of plant communities are affected by the difference of the constituent species, so that for the study of a plant community an ecological analysis of the distribution of the plant species is important.

Hitherto, the floristical and ecological distribution of broad-leaved evergreen trees and their forest in the warm temperate zone in Japan has been reported by many investigators [4-6, 11-13, 22-24, 28, 34-41]. However, the boundaries of the vegetation zone or the climatic zone which were described by these investigators were somewhat divergent and it was found that the boundary of the distribution of a certain plant species was wider than the vegetation zone which included the same species. Similar facts have been found about other plants and forests. In general, this may be due to the reason that the boundary of the vegetation has been determined by the differences in the relative quantity and the quality of the various plant species inside the plant communities and the boundary line has been drawn in a mixed forest of two communities. Besides, it was found that the boundary lines of the vegetation zones have been determined only in correlation with a single factor of environment in the neighbourhood of the zones (for example, isotherm). It may be said that the same correlative method was adopted to determine the distribution of plant species and also that such excessive factors as heat, cold and drought were selected as the controlling factors. That is, it seems that hitherto the determination of the limiting line in the distribution of plants and plant communities has been related only to a single factor in the neighbourhood of the line. Nevertheless, the determination of distribution is not so simple [47] and as was shown in a previous report by the author [21] probably is not due only to the excessive factors.

Plant distribution is seldom analysed throughout the whole vegetation period from the physiological and ecological viewpoints of dry matter production, although Boysen Jensen [2, 3] has developed his excellent idea by using deciduous trees and agricultural plants, and Tranquillini [50-52], Saeki and Nomoto [31], Pisek and Winkler [30], and Kuroiwa [15] have reported from this viewpoint.

As far as the author knows up to this time, the distribution of broad-leaved evergreen trees and their forest have not been studied on the basis of dry matter

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production. Therefore, the author investigated the light- and temperature-photosynthesis curves of fifteen broad-leaved evergreen species in Kagoshima city and in the Amami-Oshima Islands in south-western Japan and briefly analysed their distributions by the curves [17-20]. In the light-photosynthesis curves of the sun and shade-leaves, the rates of photosynthesis and respiration, the compensation point and the relative values of daily net production which were calculated from the above curves were almost proportionate to the tolerance in shade of each plant. However, because it is necessary to calculate the production of the individual plant or of the community from the ecological viewpoint, the respiration rates of stems and roots and the ratio of these organs to leaves must be measured.

In the next place, the temperature-photosynthesis curves were investigated in summer and in winter. The two curves were different and the maximum rate in summer was higher than that in winter. However, the difference between the maximum rate in summer and that in winter was smaller in the plants which are distributed in the highland or the northern region, than in those of the lowland or the southern region. From the above results, it is supposed that the activities of photosynthesis and respiration in each plant must be investigated in different seasons and in various districts, and that the production in winter is especially important for the determination of plant distribution. Afterwards, in order to explain the above problems, some data were gathered, so that in the present paper the distribution has been analysed mainly from the standpoint of the relationship between the temperature condition and the dry matter production.

## II. THE SEASONAL CHANGES OF THE PHOTOSYNTHETIC ACTIVITY IN DIFFERENT ALTITUDES, THE SEASONAL CHANGES OF RESPIRATION, AND THE TEMPERATURE CURVES OF RESPIRATION OF STEM AND ROOT

For the causal elucidation of plant distribution based on the dry matter production, it is highly important to investigate the seasonal changes of the specific physiological activities of photosynthesis and respiration under the conditions of the natural habitat.

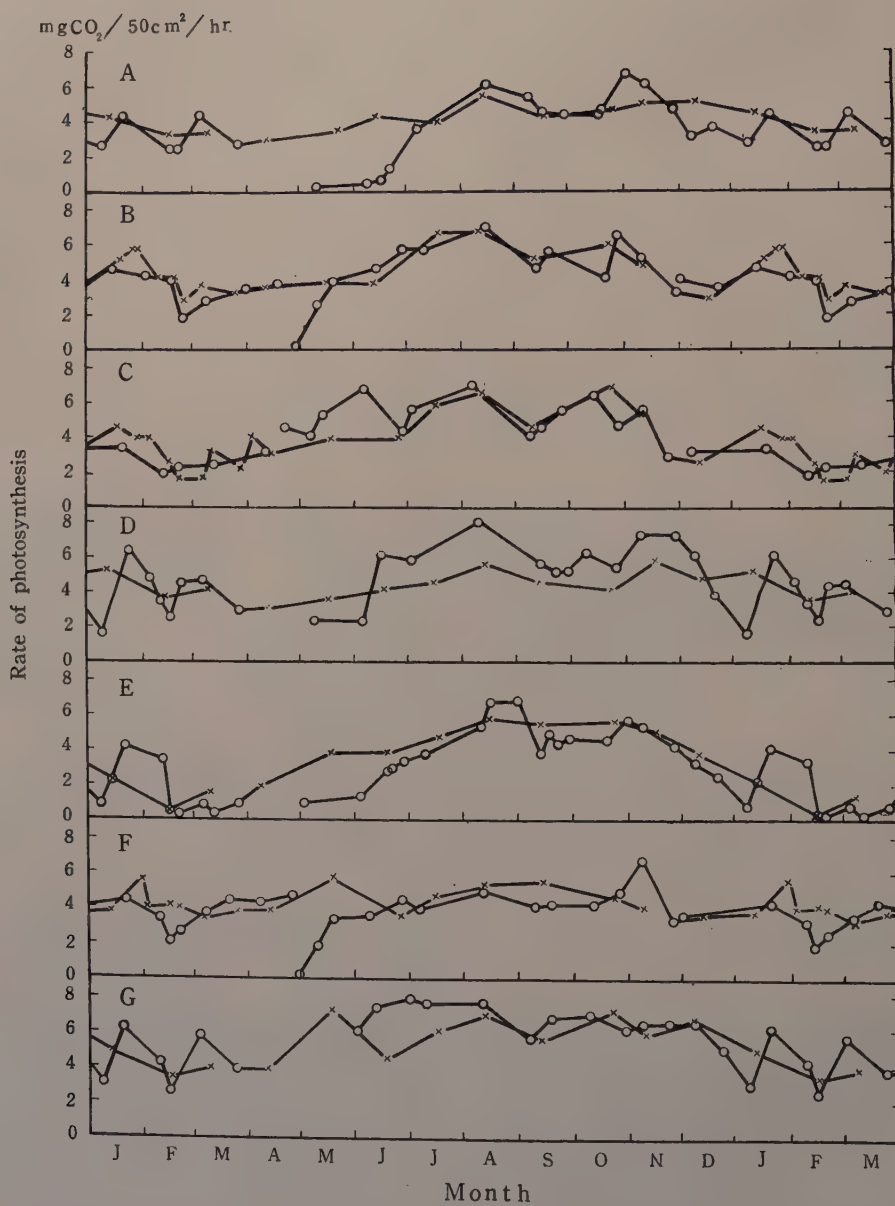
### 1) *The seasonal changes of the photosynthetic activity*

The seasonal change of the photosynthetic activity was investigated in twelve broad-leaved evergreen specieses. The activity was measured with severed leaves by Boysen Jensen's method. Most of the twelve species used were of the dominant or the characteristic species in the warm-temperate climax forest distributed in southern Japan. Four association can be recognized after Suzuki [35] and Kitazawa *et al.* [12] in the forest in the Oosumi peninsula, southern Kyushu: the *Machilus Thunbergii-Rumohra aristata* association (below 150 m. in altitude), the *Shiia Sieboldii-Rapanaea neriifolia* association (150-250 m.), the *Distylium racemosum-Illicium religiosum* association (250-700 m.) and *Cyclobalanopsis (Quercus) acuta* association (700-900 m.). Three tree species out of the twelve used for the experiments, *Machilus Thunbergii* Sieb. et Zucc., *Shiia Sieboldii* (Makino) Makino

and *Distylium racemosum* Sieb. et Zucc. are the dominant species of their respective associations. *Lithocarpus edulis* (Makino) Nakai is the characteristic species of the *Machilus Thunbergii-Rumohra aristata* association. *Rapanaea neriifolia* Mez, *Myrica rubra* Sieb. et Zucc. and *Symplocos lucida* Sieb. et Zucc. are the characteristic species in the *Shiia Sieboldii-Rapanaea neriifolia* association. Two shrub species used, *Illicium religiosum* Sieb. et Zucc. and *Eurya japonica* Thunb. are the characteristic species in the *Distylium racemosum-Illicium religiosum* association, though the latter species is also seen in the other associations. *Camellia japonica* Linn. is distributed widely in the above three association with low fidelity. *Cyclobalanopsis* (*Quercus*) *glauca* (Thunb.) Oerst. and *Cinnamomum Camphora* (Linn.) Siebold are found only in the secondary forests in sunny places of the lowland where the temperature is high enough. These twelve species also occur in the natural forests of Kagoshima (31° 30'N., about 50 m. in altitude, Table 1). In the sun leaves, which grow in the southern or the terminal sunny sides of the crowns of these tree species, photosynthesis measurements were made under the conditions of saturated light intensities of above 30 kilolux, natural CO<sub>2</sub> concentration and 25°C.

From the results of the experiments performed for four years in Kagoshima (Fig. 1), it was found that the photosynthetic activity, though it fluctuated widely, was as high in autumn as in summer, and in this respect different from the seasonal curves obtained by Saeki and Nomoto [31] in Tokyo. The activity declined gradually after the first frost and the minimum of activity was found at the middle of February, when it is the coldest of the year in Kagoshima. In spring the activity was to some degree recovered with rising temperature, though it did not reach the normal level of summer activity. The old leaves maintained a somewhat low activity until June when the new leaves acquired normal activity. Most of the old leaves fell by August. The longevity of these evergreen leaves is about one year. In the species, *Camellia japonica* and *Illicium religiosum*, which can be distributed even in the cold temperate zone, the photosynthetic activity in winter was either not much depressed or it equaled that of the summer. However, in species such as *Cinnamomum Camphora* and *Rapanaea neriifolia* which were restricted within the warm temperate and subtropical zones, the winter activity was rather low. The absolute rates of winter photosynthesis reported previously in the temperature-photosynthesis curves coincide with those in this investigation in all the species and were higher as compared with the values obtained by Saeki and Nomoto [31]. A slight depression of activity was observed in all the species late in March and early in September. This probably is due to the immaturity of young leaves and to the excessive heat in summer.

The leaves generally began to unfold in mid-March and the respiration rate of new leaves in this period was very high, i.e., about five times the maximum rate of mature leaves and settled to the normal value early in June. The photosynthetic capacity of the new leaves increased steadily and in June exceeded the rate of the old leaves of the preceding year, attaining to the maximum in August. The rate fell slightly early in September and rather fluctuated widely from the middle of that month to November, but the activity during that period was not essentially different from that in summer. In most of the plants measured, the



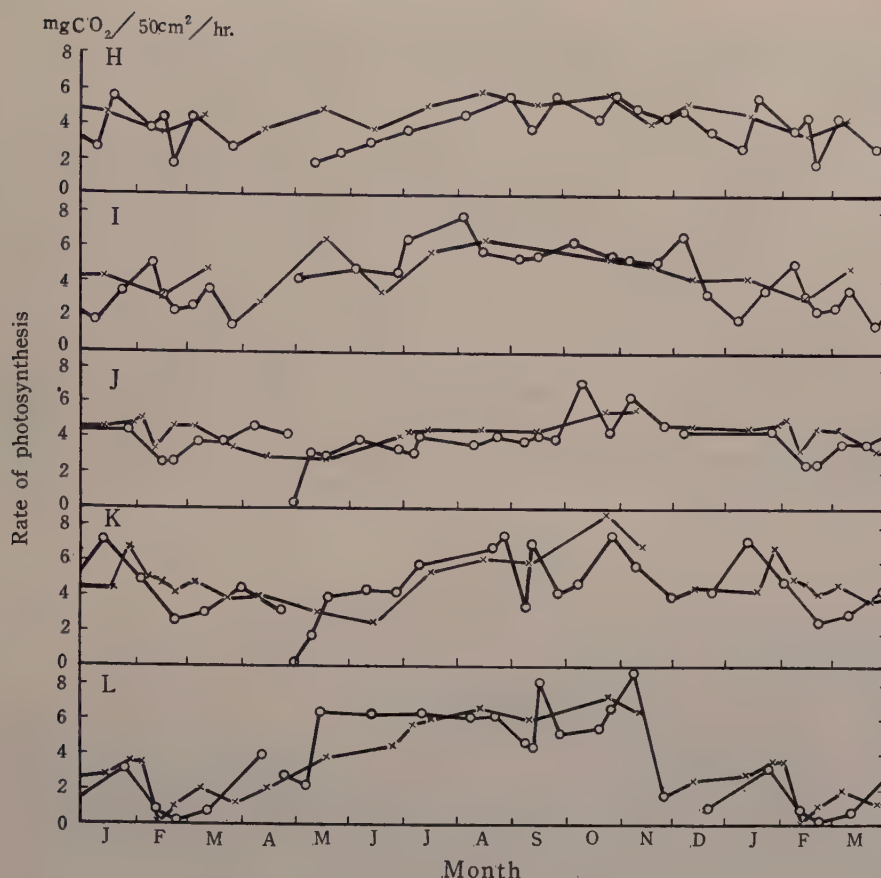


Fig. 1. The seasonal changes of photosynthesis at 25°C in Kagoshima city. The curves of B, C, F, J, K and L were investigated from December 1955, and those of A, D, E, G, H and I from May 1956. The curves of the symbol of ○ were investigated in the first year, and those of × in the second year. In the second year, for the material the new leaves were used from July.

A: *Machilus Thunbergii*  
 B: *Shiia Sieboldii*  
 C: *Distylium racemosum*  
 D: *Lithocarpus edulis*  
 E: *Rapanaea neriifolia*  
 F: *Myrica rubra*

G: *Symplocos lucida*  
 H: *Illicium religiosum*  
 I: *Eurya japonica*  
 J: *Camellia japonica*  
 K: *Cyclobalanopsis (Quercus) glauca*  
 L: *Cinnamomum Camphora*

maximum photosynthetic rate was found in summer, but in *Camellia japonica*, *Cyclobalanopsis glauca* and *Cinnamomum Camphora* it was in the autumn. The winter activity of leaves in the second year of study (1957) was higher than that of the first year probably because of the mild winter of the second year (Table 2).



TABLE 1.  
The climate of Kagoshima city and the air temperature in different altitudes in Mt. Kirishima

Districts	Altitudes m	Months	J	F	M	A	M	J	J	A	S	O	N	D
Kagoshima Meteorological Observatory	4.2	Air temp.	11.9	12.8	16.2	20.6	24.3	26.8	31.0	31.7	29.2	24.6	19.7	14.6
		Mean Max.	6.4	7.4	10.2	15.0	19.0	22.5	26.7	27.1	24.2	18.7	13.6	8.7
		Mean Min.	1.4	2.2	4.9	9.5	14.1	18.6	23.0	23.3	20.0	13.5	8.1	3.4
		Mean in the daytime	9.2	10.1	13.2	17.8	21.7	24.7	28.9	29.4	26.7	21.7	16.7	11.7
		Very fine	7	5	7	7	5	4	3	5	4	8	9	8
Kirishima Meteorological Station	240	Number of day	6	7	5	6	5	3	7	10	7	7	6	7
		Cloudy	15	13	13	14	15	15	15	13	16	13	10	11
		Rain	4	4	5	4	7	8	5	4	3	3	4	5
		Time of the daytime (mean) hrs.	149	145	183	184	197	158	227	245	203	202	176	166
		Mean Max.	7.9	9.3	13.1	17.7	21.7	23.5	26.6	27.7	25.5	21.3	16.3	11.2
in Mt. Kirishima	500	Mean	3.7	4.6	8.1	12.7	16.8	19.7	23.1	23.8	21.4	16.3	11.2	7.0
		Min. Mean	-0.5	-0.1	3.1	7.7	11.9	15.8	19.6	19.9	17.2	11.3	6.2	2.7
		Mean	3.1	4.1	6.9	11.7	15.7	19.2	23.4	23.8	20.9	15.4	10.3	5.4
	800	Mean in the daytime	5.9	6.8	9.9	14.5	18.4	21.4	25.6	26.1	23.4	18.4	13.4	8.4
		Mean	1.1	2.1	4.9	9.7	13.7	17.2	21.4	21.8	18.9	13.4	8.3	3.4
		Mean in the daytime	3.9	4.8	7.9	12.3	16.4	19.4	23.6	24.1	21.4	16.4	11.4	6.4
	1000	Mean	-0.2	0.8	3.6	8.4	12.4	15.9	20.1	20.5	17.6	12.1	7.0	2.1
		Mean in the daytime	2.6	3.5	6.6	11.2	15.1	18.1	22.3	22.8	20.1	15.1	10.1	5.1
	1500	Mean	-3.5	-2.5	0.3	5.1	9.1	12.6	16.8	17.2	14.3	8.8	3.7	-1.2
		Mean in the daytime	-0.7	0.2	3.3	7.9	11.8	14.8	19.0	19.5	16.8	11.8	6.8	1.8

TABLE 2.  
Mean air temperature of each month in winter in Kagoshima city (°C)

Months	Jan.	Feb.	Mar.	Nov.	Dec.	Average
1955				12.3	8.9	
1956	6.9	6.3	11.9	12.6	6.9	8.84
1957	8.7	7.0	9.4	14.4	9.6	9.82
1958	7.3	8.6	11.4	11.3	11.0	9.92

It is interesting that the trends of seasonal changes in photosynthetic activity are much the same with those attained by Saeki and Nomoto in Tokyo, although the winter rates in Kagoshima were higher than in Tokyo with a shorter period of the depression in the activity. The photosynthetic activity of the newly matured leaves generally equals those of herbs [16] and deciduous trees [32, 42].

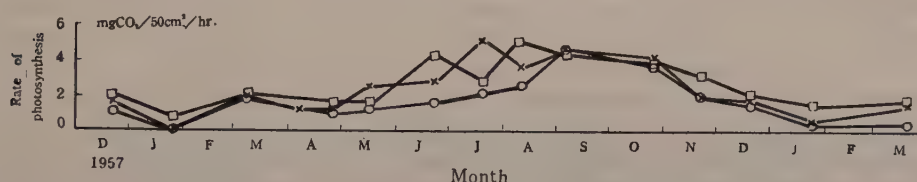


Fig. 2. The seasonal changes of photosynthesis (at 25°C) at the foot of Mt. Kirishima (about 800 m. in altitude). The new leaves were used from June 1958 for the material.

○ *Machilus Thunbergii*, × *Shiia Sieboldii*, □ *Distylium racemosum*.

These results for *Machilus Thunbergii*, *Shiia Sieboldii* and *Distylium racemosum* obtained in Kagoshima were compared with the data gained in a colder region of high altitude, the foot of Mt. Kirishima (the peak is 1700 m. in altitude). The station is situated at 50 km. in a north-eastern direction away from Kagoshima city and its altitude is about 800 m. above sea level, just below the altitudinal limit of the broad-leaved evergreen forest in this district. The measurements of photosynthesis were executed with a portable apparatus, improved from the Boysen Jensen apparatus, under the same conditions as in Kagoshima. The seasonal photosynthetic curves obtained are shown in Fig. 2 and the air temperatures at the foot of Mt. Kirishima in Table 1. The air temperatures in this table at 500, 800, 1,000 and 1,500 metre's altitude were calculated with a lapse rate of 6.6°C per 1,000 m. on the basis of the air temperature at the Kagoshima Meteorological Observatory (4.2 m. above sea level) as a standard. The values are somewhat higher in comparison with those at the Kirishima Meteorological Station (240 m. in altitude) near the experimental station—the lapse rate calculated between Kagoshima and Kirishima was rather too high, i.e. 10°C (7.7°C in Mt. Takakuma [12]). The curves in Fig. 2 were more or less the same as those in Fig. 1, although the photosynthetic rate in summer was slightly smaller at

Kirishima than in Kagoshima. These results show a similar trend to the seasonal change in photosynthetic activity of the conifers growing in a highland of Europe determined by Pisek and Winkler. Because of the delay caused by lower temperature the unfolding of leaves at the station of Mt. Kirishima began early in May which is about one month and a half later than the time of unfolding in Kagoshima (Table 1). The increasing trends of the photosynthetic activity of new leaves were more or less the same at both districts. The high photosynthetic activity in summer was maintained in Kagoshima city for about six months, from the middle of May to the middle of November, but only three or four months at the station of Mt. Kirishima (about five months in Tokyo). The maximum activity in summer was in the range of 4.8~5.8 mg. CO<sub>2</sub>/50 cm.<sup>2</sup>/hr., being 1.5~2.0 mg. lower than those in Kagoshima. The winter minimum of the photosynthesis appeared in January at Kirishima, while in February in Kagoshima. The value at Kirishima was 0 mg. CO<sub>2</sub>/50 cm.<sup>2</sup>/hr. in *Machilus Thunbergii* and *Shiia Sieboldii*, and 0.8 mg. in *Distylium racemosum*. Throughout the winter, the photosynthetic rates at Kirishima were as low as one half, in *Shiia Sieboldii* and *Distylium racemosum* and one third, in *Machilus Thunbergii* of the values obtained in Kagoshima. These depressed photosynthetic activities were seldom recovered, and the old leaves were replaced by new leaves with normal activities as seen in Kagoshima. As mentioned above, it seems that the curve showing seasonal change of photosynthetic activity in Tokyo may run between the curves of Kagoshima and Kirishima, because the temperatures in Tokyo (3.1°C mean temp. in January) correspond to those at the place of 500 m. in altitude. It may be presumable that the nearer to the upper or northern limit of the distribution, the more the dry matter production of the evergreen trees decreased in winter on account of the lower temperature, the period of low production being prolonged.

## 2) The seasonal changes of respiration of leaf, stem and root

Sun leaves and the branches bearing them were used for measurement of respiration, which was performed by Boysen Jensen's method at 25°C in Kagoshima city. The results are shown in Figs. 3, 4 and 5. The seasonal changes of the respiration of leaves showed a similar trend in all the species investigated. The respiration of new leaves was about four times that of normal leaves and the duration of the high respiration was about three months, from the end of March to the middle of June. The respiration of new leaves was much higher in *Lithocarpus edulis*; *Cyclobalanopsis glauca* and *Rapanea neriifolia* and lower in *Illicium religiosum* than in the other plants. Although the respiration of mature leaves in all the plants decreased slightly in winter, no remarkable changes were generally found throughout the year.

The seasonal changes of respiration in stems (branches) and roots were almost the same as the trends of those of leaves and no remarkable difference was found between the species. The respiration of new stems was higher than that of the matured stems and the periods of high respiration were also about three months. From the seasonal trends of respiration of leaves, stems and roots, it may be concluded that under the condition of a constant temperature little changes are expected in the respiration rate of these plant organs measured

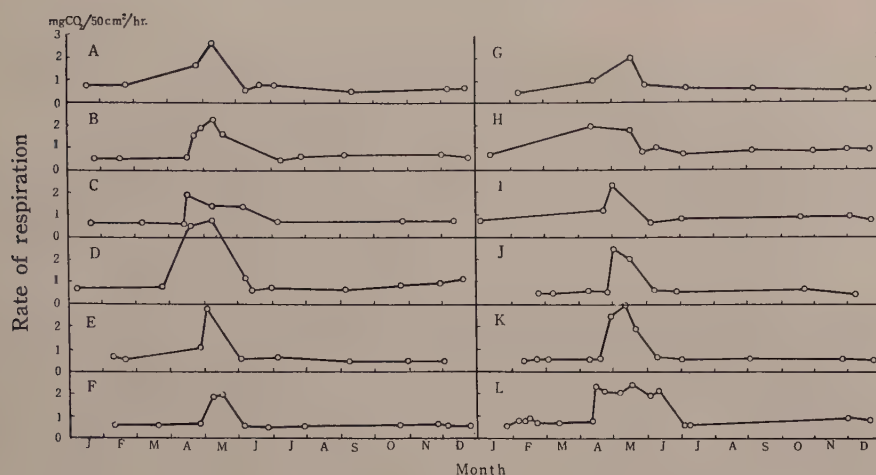


Fig. 3. The seasonal changes of respiration of leaf at 25°C in Kagoshima city. The period of the beginning of investigation in each plant and the symbols of A, B, C, etc. are the same as in Fig. 1, and for the symbols, the same rule, applies to the following figures.

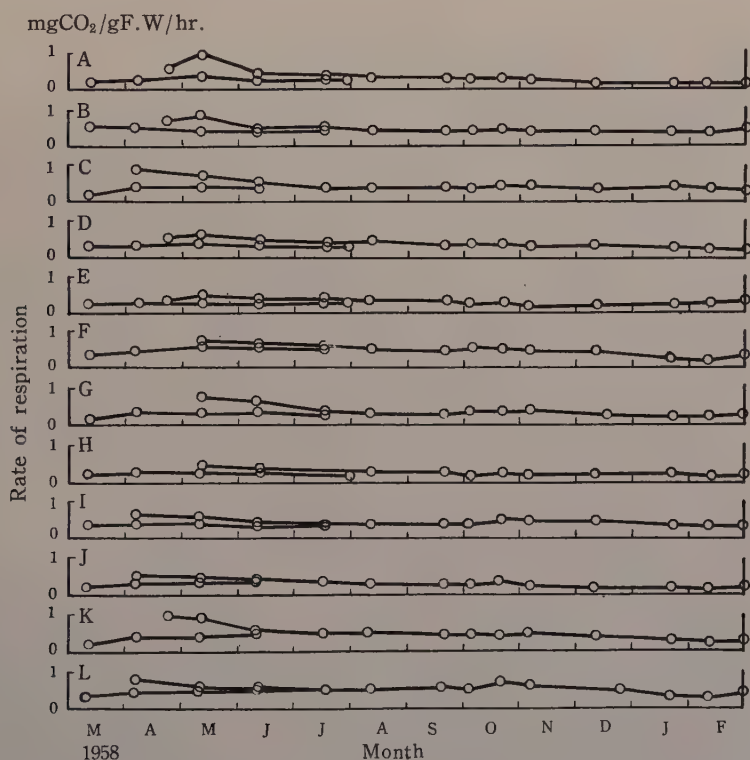


Fig. 4. The seasonal changes of respiration of stem at 25°C in Kagoshima city. Those of the new stems were investigated in April, May, June and July.



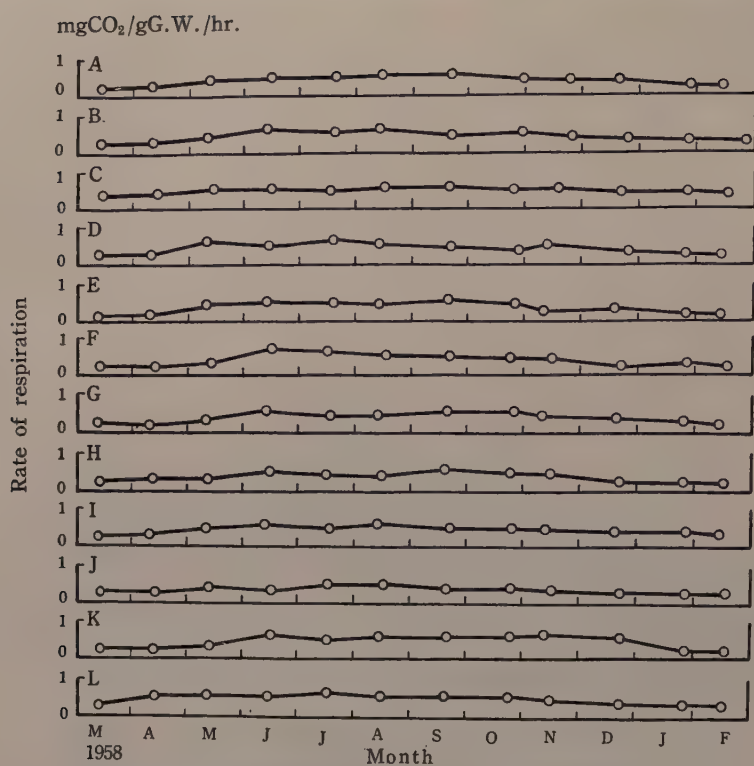


Fig. 5. The seasonal changes of respiration of root at 25°C in Kagoshima city.

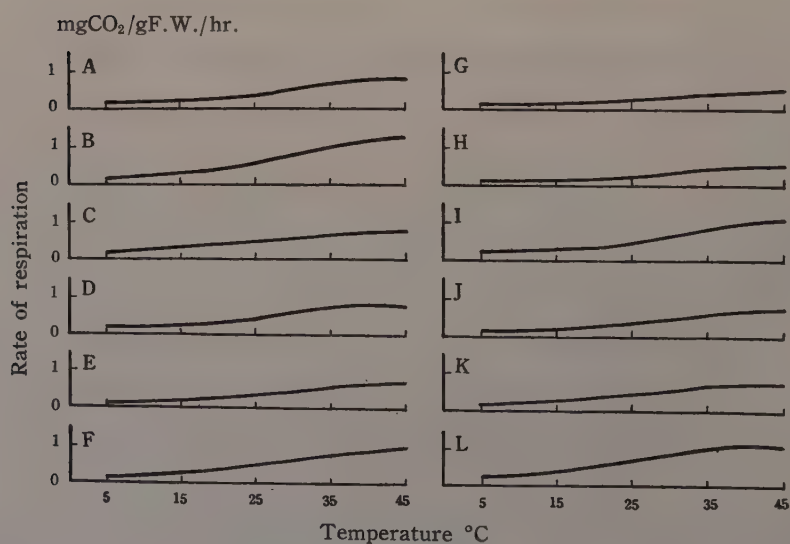


Fig. 6. The temperature-respiration curves in stem.

in different seasons and in different altitudes, and that the temperature variation is the only determining factor for the respiration rate in the field.

### 3) The temperature-respiration curves of stem and root

The temperature-respiration curves of stem (Fig. 6) and root (Fig. 7) were made in summer in Kagoshima. Although the curves were obtained from sun and shade branches after excising the leaves, only the curves for sun branch

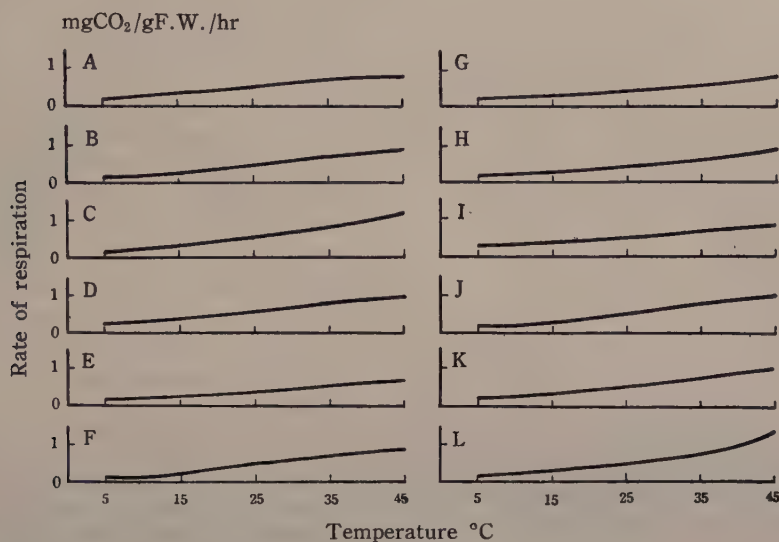


Fig. 7. The temperature-respiration curves in root.

are shown in Fig. 6. The respiration rate was slightly higher in the sun branch than in the shade one in high temperature, but the difference was hardly found in low temperature. Every curve in stem and root rose with increasing temperature sharply at 25°C as seen in the temperature-respiration curves of leaves in the previous papers. The respiration rate in absolute value was lower in stems than in leaves. The value in the root was higher than that in the stem at the temperature below 30°C.

## III. THE ACTIVITY OF PHOTOSYNTHESIS AND RESPIRATION IN *CYCLOBALANOPSIS ACUTA* THUNB.

*Cyclobalanopsis acuta* is a dominant species in the broad-leaved evergreen forest near the upper limit (700~900 m. in altitude) in Kagoshima Prefecture. The lower limit of natural distribution of this plant is about 300 m. in altitude in Kagoshima city, though it actually grows by transplanting in the lowland below this lower limit. Such limited distribution should be studied on the basis of dry matter production. Although it is desirable that the photosynthetic activity will be investigated at the centre of distribution (for *C. acuta* about 800 m. in Mt. Kirishima), the measurements of photosynthesis and respiration on the

outside of natural distribution may also give important information for elucidation of the problem concerned. The materials were collected from the thirty-year-old trees transplanted in the Botanical Garden of the Faculty of Agriculture, University of Kagoshima, Kagoshima city.

The light-photosynthesis curves in the sun and shade leaves at 25°C are shown in Fig. 8. The curves indicate relatively high photosynthetic rate, com-

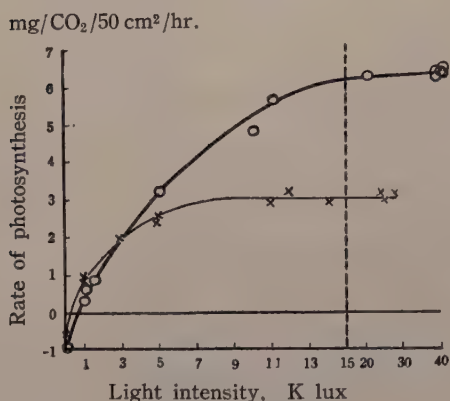


Fig. 8. The light-apparent photosynthesis curves at 25°C in sun and shade leaves of *Cyclobalanopsis (Quercus) acuta*. The thick line (○) is of sun leaf and the thin line (×) of shade leaf.

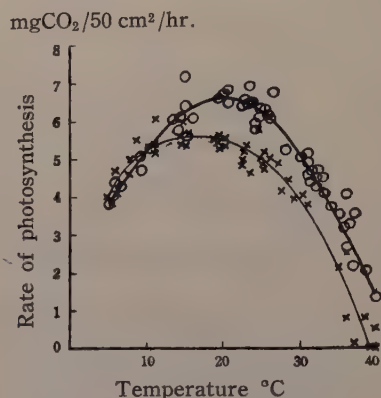


Fig. 9. The temperature-apparent photosynthesis curves at light intensities above 30 kilolux in summer and winter in *Cyclobalanopsis (Quercus) acuta*. The thick line (○) shows the curve in summer and the thin line (×) in winter.

photosynthesis point and respiration rate; this may suggest that *Cyclobalanopsis acuta* has physiological characters of semi-shade trees as well as *Distylium racemosum*, *Cinnamomum Camphora* and *Ardisia Sieboldii*. The temperature-photosynthesis curves in Fig. 9 obtained in saturated illumination (above 30 kilolux) illustrate that the highest value of photosynthesis occurs at about 20°C in summer, and at about 15°C in winter, and that the optimum temperature in this species is lower than those of the twelve plants growing in the lowland which were described before. Furthermore, the rate of photosynthesis in Fig. 8 might become higher, if measured at 20°C. It is very interesting that the results obtained correspond to those of the conifers growing in the highland in Europe measured by Tranquillini [50-52], and Pisek and Winkler [30]. This fact will imply that the genetic character of the highland plant is not easily varied by such a change of environment as transplanting to the lowland. This has also been proved previously by the author on the basis of the temperature-photosynthesis curves of some tropical and subtropical plants in the Amami-Oshima Islands in south-western Japan.

The temperature-respiration curves in the sun leaves, stems and roots are shown in Fig. 10. The curves in stems and roots of *Cyclobalanopsis acuta* were not greatly different from those of the twelve other plants, but the respiration curves of the sun and shade leaves rose steeply at temperatures above 15°C.

It is therefore anticipated that the respiratory consumption increases and conversely that the dry matter production decreases in the lowland with high temperature. The seasonal changes of activity of photosynthesis of the sun leaves and of respiration of sun leaves, stems and roots are illustrated in Figs. 11 and 12. Each curve was obtained at 25°C; the net photosynthesis would be somewhat higher, if measured at 20°C, the optimum temperature. The photosynthetic activity scarcely changed throughout the year (Fig. 11), except that it fell slightly in January. The slight declines in April and August and the fluctuation in autumn are similar to those of the twelve other species in Kagoshima city. Unfolding of leaves begins early in May at the distribution limit (in Kagoshima city), and it is the same as the distribution centre and one month and a half later than those of the plants in the lowland. The photosynthetic activity of new leaves settled rapidly to that of the normal mature leaves. On the whole, the steady course throughout the year of *Cyclobalanopsis acuta* is very different from those of the lowland species, except for *Camellia japonica* and *Illicium religiosum*.

No large change in the activity of respiration of the mature leaves was found in each season (Fig. 11). The respiration of new leaves was about twice that of the normal leaves, the duration of such high respiration being about two

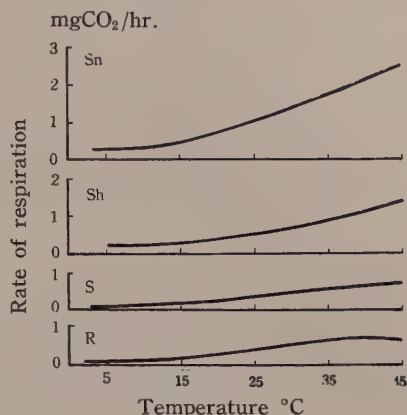


Fig. 10. The temperature-respiration curves of sun (Sn) and shade leaves (Sh), stem (S) and root (R) in *Cyclobalanopsis (Quercus) acuta*. The rates of sun and shade leaves are shown per 50 cm<sup>2</sup>. of leaf area, and those of stem and root per g. of fresh weight.

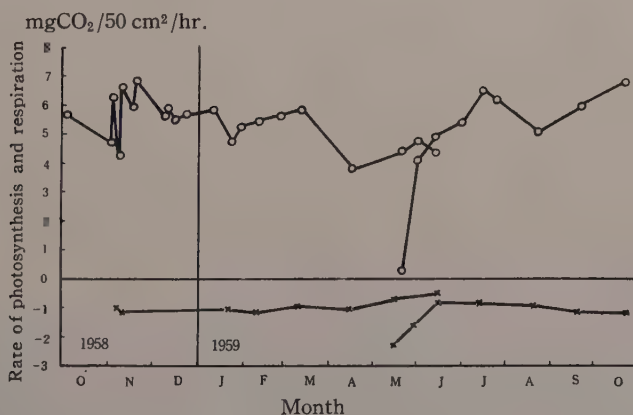


Fig. 11. The seasonal changes of photosynthesis and respiration of *Cyclobalanopsis (Quercus) acuta* at 25°C in Kagoshima city. (○) Photosynthesis, (×) respiration of leaf.





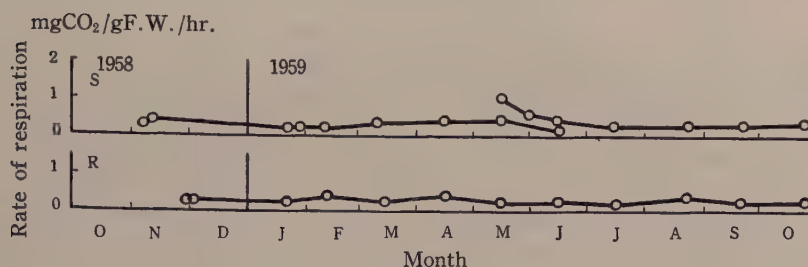


Fig. 12. The seasonal changes of respiration of stem and root in *Cyclobalanopsis (Quercus) acuta* at 25°C in Kagoshima city. S: stem, R: root.

months. The seasonal curves of respiration activity of the stem and root change little throughout the year, though they depressed somewhat in winter (Fig. 12).

In conclusion, the facts that in *Cyclobalanopsis acuta* the photosynthetic activity did not much fall even at a low temperature and the respiration was especially high at high temperature, seem to be the main reasons why this species is able to distribute in the highland in contrast to the other broad-leaved evergreen trees growing in the lowland. The low temperature of the highland may affect advantageously the distribution of *Cyclobalanopsis acuta* because of higher matter production.

#### IV. THE DRY MATTER PRODUCTION IN THE YOUNG BROAD-LEAVED EVERGREEN TREES

Young trees were used for the experiment for the following reasons. In order to calculate the net production per individual plant, it is necessary to obtain the C/F-ratio (ratio of nonphotosynthetic system to photosynthetic system [7]) and the collecting of young trees was easier than that of old large trees. Kitazawa *et al.* [11] has determined the C/F ratio in the old trees most recently. Moreover, competition starts at the seedling stage under natural conditions, so that if the environment at this stage is favourable for one tree species its matter production must be high and the tree species may grow up more quickly than other species and dominate over the latter.

Young trees of the thirteen species described above were collected widely from Kagoshima Prefecture in two periods, i.e., about a month after the unfolding stage in spring and at the beginning of autumn when the fall of old leaves ended. The young trees used were from one to five years old. In each, the plant was separated into the leaf blade, stem and root to measure their fresh and dry weights. In Table 3 are presented the values of the ratio of stems to leaves and those of roots to leaves, respectively. The T/R ratios (ratio of top to root) of the seedlings were generally in the range from one to four [1], which coincide with the values hitherto measured.

The production was calculated on the basis of the temperature in different altitudes in Table 1 and the weight ratio of organs in Table 3, using the tem-

perature curves and the seasonal changes of photosynthesis and respiration illustrated before. Here it was presumed that the photosynthesis of all the leaves was saturated with the full sunlight. Although in previous papers [18-20] the daily production at various temperatures in summer and winter was presented as production by leaves alone (surplus production), in this paper the daily gross production of leaves was in the first place calculated per individual plant, and then the daily respiration per individual plant. The daily net production in an individual plant is the difference between the gross production and the total respiration. An example based on the data for *Cyclobalanopsis acuta* is shown in Fig. 13. The results for other species are derived from the temperature curves presented in the previous papers (Fig. 14). The calculation was made as follows. The daily gross production and daily respiration were expressed as a relative value, as presented in Figs. 13 and 14. To obtain the daily gross production curve, this was multiplied by twelve in summer curve and by eight in winter

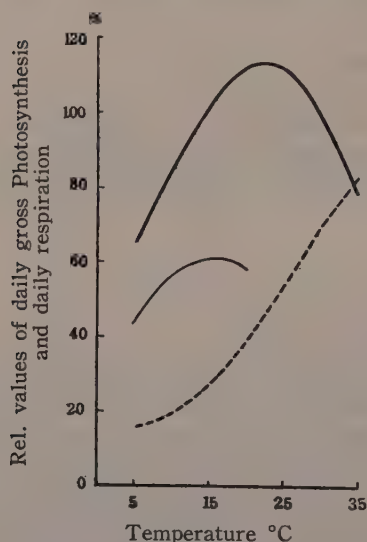


Fig. 13. The relative values of daily gross photosynthesis and daily respiration per individual plant at various temperatures in *Cyclobalanopsis (Quercus) acuta*. The thick line shows the curve in summer, the thin line in winter, and the broken line of the respiration.

multiplied by 10/8, and in the case of use of the summer curve, by 10/12, as the assimilation hours are assumed as 10. Gross production in a day or a month is read from the curves in Figs. 13 and 14 against the mean monthly temperatures in the daytime (mean between the maximum air temperature and the mean air temperature in each month), and the respiration against the monthly mean temperature.

curve, assuming that the assimilation hours were 12 in summer and 8 in winter. The relative value of total daily respiration per plant was calculated on the basis of the values of ratio of each organ and 24 hours of respiration. For the calculation of annual production, the assimilation hours were assumed as 12 in May, June and July, 8 in November, December and January, and 10 in February, March, April, August, September and October, so the annual total of the assimilation hours was 3650. However, from Table 1 the rainy days in Kagoshima are on the average five in a month, so that the amount of light usable for photosynthesis is about 80 per cent of the annual total assimilation hours. In the calculation of the production in the six months of spring and autumn, the winter curve should be employed for the months with frost, while the summer curve for the months without frost. The former is adopted in this paper for the months with a mean temperature below 10°C or with a minimum mean temperature below 5°C. In this case the value read from the winter curve is

In order to obtain the annual net production per individual plant at each altitude, the daily net production must be summed up according to monthly air temperature of each altitude. This calculation is very complicated, so that the curves of relative values of gross production and respiration in Figs. 13 and 14 are represented by the following formula:

$$y = at^2 + bt + c.$$

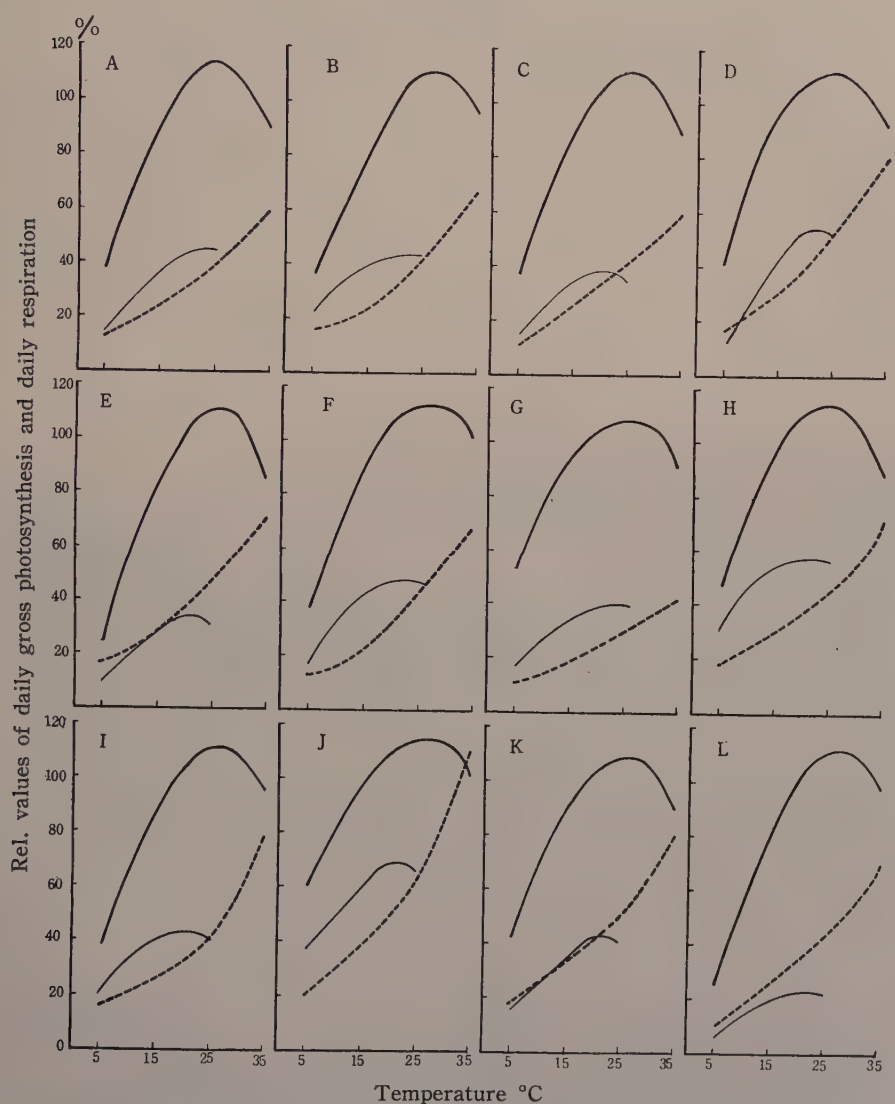


Fig. 14. The relative values of daily gross photosynthesis and daily respiration per individual plant at various temperatures in the twelve species of Fig. 1. The thick, thin and broken lines for each species indicate the same as in Fig. 13.



TABLE 4.  
 Constants in the formula obtained on the basis of the curves in Figs. 13 and 14

Plant species	Photosynthesis curves					Respiration curves		
	in Summer		in Winter			a <sub>3</sub>	b <sub>3</sub>	c <sub>3</sub>
a <sub>1</sub>	b <sub>1</sub>	c <sub>1</sub>	a <sub>2</sub>	b <sub>2</sub>	c <sub>2</sub>			
<i>Machilus Thunbergii</i>	-0.13	7.8	1		1.8	7	1.2	7
<i>Shiia Sieboldii</i>	-0.09	6.2	8	-0.10	4.0	3	-0.1	12
<i>Distylium racemosum</i>	-0.16	8.7	-4	-0.02	2.2	5	1.5	4
<i>Lithocarpus edulis</i>	-0.19	9.2	2		2.8	0	1.7	7
<i>Rapanea nerifolia</i>	-0.19	10.0	-24		1.6	2	1.6	6
<i>Myrica rubra</i>	-0.17	9.1	-7	-0.12	5.2	-6	0.05	12
<i>Symplocos lucida</i>	-0.13	6.6	25	-0.05	2.6	7	1.0	4
<i>Illicium religiosum</i>	-0.18	8.8	8	-0.10	4.3	13	1.2	13
<i>Eurya japonica</i>	-0.15	8.0	2	-0.11	4.4	-1	1.2	10
<i>Camellia japonica</i>	-0.11	6.2	28		2.1	27	2.0	10
<i>Cyclobalanopsis glauca</i>	-0.14	7.4	10		1.9	8	1.6	8
<i>Cinnamomum Camphora</i>	-0.20	10.2	-22	-0.06	2.6	-6	1.8	2
<i>Cyclobalanopsis acuta</i>	-0.18	7.8	22	-0.27	7.7	9	-0.1	15

TABLE 5.  
Photosynthesis and respiration of leaf summarized from previous papers, and respiration of  
other organs summarized from the present paper

Plant species	Rates of apparent photosynthesis mgCO <sub>2</sub> /50 cm <sup>2</sup> /hr.		Rates of respiration mgCO <sub>2</sub> /g fresh weight/hr.				
	in Summer at 25°C	in Winter at 20°C	at 25°C		at 15°C		
			Leaf	Stem	Root	New leaf	New stem
<i>Machilus Thunbergii</i>	4.80	2.65	1.51	0.31	0.50	0.85	0.45
<i>Shiia Sieboldii</i>	5.50	3.10	0.94	0.52	0.44	0.85	0.57
<i>Distylium racemosum</i>	5.80	2.90	0.75	0.48	0.55	0.88	0.56
<i>Lithocarpus edulis</i>	6.10	4.50	0.81	0.40	0.53	0.69	0.27
<i>Rapanaea neriifolia</i>	4.70	2.05	0.39	0.33	0.38	0.51	0.31
<i>Myrica rubra</i>	4.60	2.90	0.86	0.50	0.54	1.05	0.46
<i>Symplocos lucida</i>	6.80	3.50	0.55	0.32	0.42	0.64	0.40
<i>Illicium religiosum</i>	4.60	3.40	0.65	0.23	0.40	0.52	0.24
<i>Eurya japonica</i>	5.70	3.20	0.72	0.40	0.50	0.52	0.34
<i>Camellia japonica</i>	4.20	3.85	0.55	0.34	0.50	0.53	0.31
<i>Cyclobalanopsis glauca</i>	6.00	3.45	0.76	0.48	0.53	1.05	0.66
<i>Cinnamomum Camphora</i>	6.20	1.55	0.93	0.63	0.51	1.10	0.51
<i>Cyclobalanopsis acuta</i>	6.30	5.00	0.94	0.40	0.40	1.20	0.61

In this formula,  $y$  is the relative value of daily gross production or daily respiration of the individual plant,  $t$  is air temperature of daytime in the case of photosynthesis, and the monthly mean air temperature in the case of respiration, and  $a$ ,  $b$ ,  $c$  are constants, characteristic of each plant species as shown in Table 4.

Using the accumulated temperature, the relative value of the annual net production ( $30 \times Y$ ) was calculated by the following equation,

$$30 \times Y = (a_1 \sum t_1^2 + b_1 \sum t_1 + \sum c_1) + (a_2 \sum t_2^2 + b_2 \sum t_2 + \sum c_2) - (a_3 \sum t_3^2 + b_3 \sum t_3 + 12 \times c_3),$$

where the first term in the right side of the equation corresponds to the gross production value in the warmer season, the second, that in the colder season with frost, and the third the annual total of respiration,  $t_1$ ,  $t_2$  and  $t_3$  are the mean air temperature of daytime in warmer and colder seasons, and the mean air temperature, respectively. To obtain the annual value,  $Y$  must be multiplied by the average thirty days of a month.

The calculated relative values of the annual net production per individual plant in different altitudes are shown in Fig. 15. The annual net production

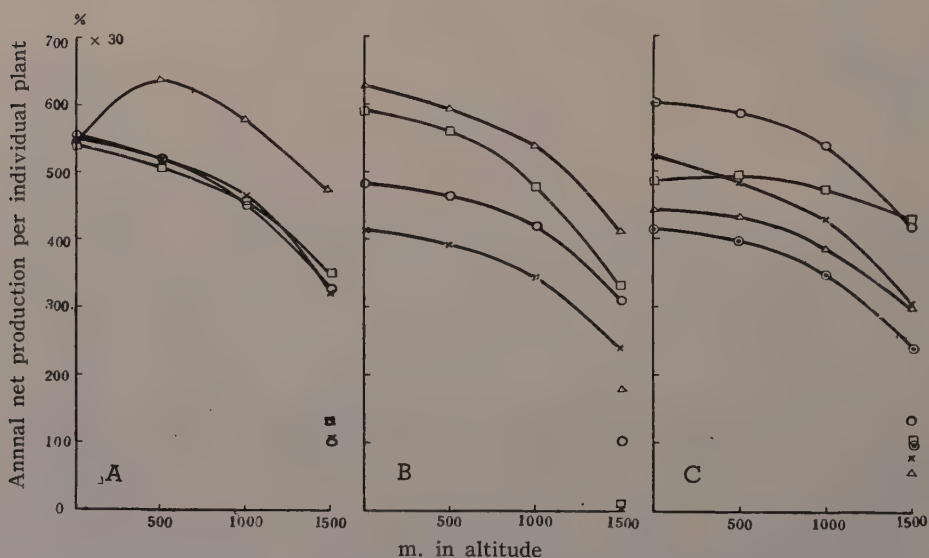


Fig. 15. The relative values of annual net production per individual plant in different altitudes. As to the marks at lower part of 1500 m. see the text.

- |  |   |
|--|---|
| A. ○ <i>Machilus Thunbergii</i>          | △ <i>Symplocos lucida</i>                 |
| × <i>Shiia Sieboldii</i>                 | C. ○ <i>Illicium religiosum</i>           |
| □ <i>Distylium racemosum</i>             | × <i>Eurya japonica</i>                   |
| △ <i>Cyclobalanopsis (Quercus) acuta</i> | □ <i>Camellia japonica</i>                |
| B. ○ <i>Lithocarpus edulis</i>           | △ <i>Cyclobalanopsis (Quercus) glauca</i> |
| × <i>Rapanaea neriifolia</i>             | ▷ <i>Cinnamomum Camphora</i>              |
| □ <i>Myrica rubra</i>                    |   |

values of every plant species decreased in proportion to rising altitudes except for *Cyclobalanopsis acuta* and *Camellia japonica* which have the maximum value at about 500 m. in altitude. However, the degree of decrease was different between plant species, viz. the decreasing degree in *Illicium religiosum*, *Symplocos lucida*, *Cyclobalanopsis glauca*, *Lithocarpus edulis* and *Distylium racemosum* was low; in *Myrica rubra*, high, and in the other plants, moderate. It is very interesting that such species as *Cyclobalanopsis acuta* and *Camellia japonica*, which can distribute in the district of lower temperature, have the ability of higher production in highland of about 500 m.

The fact that some positive production was calculated in all plant species even at 1,500 m. in altitude which is higher than the upper limitation of natural distribution may be caused by dealing only with a single factor such as temperature. Nevertheless the period of high respiration in new leaves and new stems of three months (Figs. 3 and 4, Table 5), the decrease of light saturation hours to 80 per cent of the theoretical value by clouding (see p. 322) and the low productivity, especially in winter at highland (see p. 313), are all taken into consideration, and it gives the much lower productivity shown by the values at the bottom in Fig. 15. Further consideration of the many other factors than temperature, e.g. drought factor in winter, can illustrate more precisely the real distribution of these plants.

## V. DISCUSSION

Although the northern or upper limit of distribution of broad-leaved evergreen trees may generally be determined by the effect of excessive cold in winter, it seems to be not always applicable to every tree species, because some of these trees could resist even the cold temperature [21]. The limitation of the distribution may therefore be elucidated to a great extent from the standpoint of the dry matter production in the vegetation period. The outermost limitation should appear at the place where the plants cannot perform any positive matter production. This theoretical distribution—Boysen Jensen's potential distribution [3]—must be wider in comparison with the natural distribution range as it is shown in Fig. 15. One of the causes of such difference might be due to the fact that only the temperature factor is considered in the calculation of matter production under the optimal condition of other factors. If the effect of light factor on the photosynthesis is considered together with the temperature factor, the distribution of each species may be explained more accurately. From the curves in Fig. 15 and the light-photosynthesis curves in the previous paper [19], the following may be said. Among *Machilus Thunbergii*, *Shiia Sieboldii* and *Distylium racemosum*, dominant species of the corresponding associations, the production is favourable in the lowland for *Machilus Thunbergii* which is very tolerant in shade, while the production in *Distylium racemosum*, the semi-shade tree, is slightly higher than in *Shiia Sieboldii* under the conditions of low temperature, *Distylium racemosum* constitutes its community in the highland, where it is rather unfavourable for *Machilus Thunbergii* and *Shiia Sieboldii* because of the low temperature, and *Distylium racemosum* can receive more sufficient light by



the decline of the competing plants. Comparing the matter production of these three species, it is conceivable that the *Shiia Sieboldii* association develops in the district between both the associations. From the relation between altitude, light and matter production, it appears also natural that *Cyclobalanopsis acuta* dominates in the area beyond the upper district where *Distylium racemosum* dominates. Characteristic species such as *Lithocarpus edulis*, *Myrica rubra*, *Rapanaea neriifolia* and *Illicium religiosum* can grow respectively in the communities in the lowland and highland according to characteristics of matter production for the temperature [18] and light factors [19]. The causes why *Camellia japonica* and *Eurya japonica* can distribute widely in every association may be explained by the curve in Fig. 15. *Cyclobalanopsis glauca* and *Cinnamomum Camphora* are favourable in the lowland in matter production. *Symplocos lucida*, a characteristic species in *Shiia Sieboldii*-*Rapanaea neriifolia* association can perform higher matter production under the conditions of weak light intensity and low temperature, so that the wider distribution of this species may be expected. The alternation of the dominant species in this secondary forest can be explained by the same manner [46].

In this paper, only the temperature factor is dealt with under the optimal conditions of the other factors, but if both the effects on matter production of various factors such as light [9, 26, 27], water [48, 49], carbon dioxide [25] and mineral nutrient [43-45], and the interspecific competitions for those environmental factors are considered additionally, it may be suggested that the theoretical limitation of the distribution of these plants can approach their natural distribution. The horizontal and vertical distributions of plants and plant communities appear to be able to be analysed fundamentally from the standpoint of dry matter production.

Monsi and co-workers [7, 8, 9, 14, 26, 27, 29, 33] have achieved extensive analyses on the relationships between plant communities and light factor. If their information is applied to the results obtained, the plant distribution problem concerned may be elucidated furthermore from the viewpoint of matter production. Also, the differences in matter production between broad-leaved deciduous and the coniferous forest, which were discussed in their papers, may be useful for analysing the establishment of the mixed forests in the neighbourhood of distribution limit. There should be made the analyses of interspecific competition for the environmental factors between the communities which consist of the trees with different life forms. This is undoubtedly one of the most important problems that must be studied in the future.

## SUMMARY

1. The seasonal changes of the activities of photosynthesis and respiration were studied in Kagoshima in twelve characteristic species of the broad-leaved evergreen forests of southern Japan, *Machilus Thunbergii*, *Shiia Sieboldii*, *Distylium racemosum*, *Cinnamomum Camphora*, *Lithocarpus edulis*, *Cyclobalanopsis (Quercus) glauca*, *Symplocos lucida*, *Myrica rubra*, *Rapanaea neriifolia*, *Eurya japonica*, *Camellia japonica* and *Illicium religiosum*. Out of them, *Machilus*

*Thunbergii*, *Shiia Sieboldii* and *Distylium racemosum* were measured in photosynthetic activity at the foot (800 m. in altitude) of Mt. Kirishima, too.

2. In winter, the photosynthetic activity decreased at the highland station to the values from a half to one third of those measured in Kagoshima. The period of the high productivity in summer was six months in Kagoshima, while it was about three months at the foot of Mt. Kirishima. The respiration rates of stems and roots decreased slightly in winter in Kagoshima. The respiration of new leaves and new stems was very high.

3. In *Cyclobalanopsis (Quercus) acuta*, a dominant species of the broad-leaved evergreen forest of the highland in southern Japan, the light- and the temperature-photosynthetic curves, the respiration curves and the seasonal changes of photosynthesis and respiration were investigated in Kagoshima. This species had a character of semi-shade tree and its winter productivity hardly suffered from low temperature.

4. The relative values of the daily matter production per individual of young trees of these thirteen species were calculated. Relationship between net production and mean air temperature was expressed with an empirical equation, after which the annual production of each species in different altitudes was calculated on the basis of its productivity and monthly mean temperature (Fig. 15).

5. The limit of plant distribution deduced from the calculated matter production is somewhat different from the natural distribution. However, the other factors along with the temperature being considered, the former roughly coincides with the latter. This may suggest that the horizontal and vertical distribution of plant communities can theoretically be elucidated by analysing the dry matter production of plants.

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## STUDIES ON THE HALOPHILIC CHARACTERS OF THE STRAND DUNE PLANTS AND OF THE HALOPHYTES IN JAPAN

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### INTRODUCTION

From the ecological point of view, strand dune plants as well as halophytes have been studied for a long time by many investigators. The strand dune soils were examined in salt content by Kearney [14], Olson-Seffer [20], Stocker [28], Kooper [16], Montfort and Brandrup [18], Benecke and Arnold [6], Arnold and Benecke [1], and Hara [10], and in hydrogen-ion concentrations by Salisbury [21, 22], Kurz [17], and Montfort and Brandrup [18], while the soil conditions in saline habitats were investigated by Steiner [27], Arnold [2], Schratz [24], Schratz and Beiler [25], and Hatanaka [12]. The strand dune plants and halophytes were studied as to their osmotic values and NaCl contents by Suzuki [29], Volk [41], Harris [11], Steiner [27], Walter and Steiner [43], Walter [42], Hori [13], Sen-Gupta [26], Takada [30-33], Ashby and Beadle [3], and Beadle *et al.* [4]. Furthermore, experimentations concerned with the effect of salt on the seed germination and the growth of the strand dune plants were carried out by Birger [8], Yoshii [47], Benecke [5], and Bickenbach [7], and those concerned with halophytes, by Halket [9], Keller [15], Montfort and Brandrup [18, 19], and Schratz [23]. As a result of these studies it is conclusive that extremely high salinity is prevailing in the soils where halophytes grow, and the osmotic values and NaCl contents of the expressed saps from the halophytes are also remarkably high, and halophytes grow better in the presence of certain amount of salt in the culture solution.

In order to investigate the halophilic characters of the strand dune plants in Japan and to find out the difference in the halophilic characters between those plants and true halophytes, the writer conducted ecological studies on the soil conditions (especially NaCl contents and hydrogen-ion concentrations), osmotic values and NaCl contents of the plant saps, and on the effect of salt on the germination and growth of the dune plants and halophytes [35-40].

Reviewing these results and new results obtained by the later researches conducted on the same line, the present writer could recognize clear differences in the halophylic characters between the strand dune plants and the halophytes; the former are much less strong in the halophilic characters than the latter, or they belong rather to mesophytes.

The conditions of the strand dune soils were investigated on the seashore of Taito (18 in Fig. 1), Chiba Prefecture, on the seashore of Usa (2) and at the river-mouth of the Niyodo River (3), Kôchi Prefecture, and on the seashore in the vicinity of Kanazawa and in the Noto Peninsula, Ishikawa Prefecture (11, 13 and 14). The conditions of the soils which were covered with the halophytic

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Fig. 1. Map showing localities of the investigated stations.

- 1, Obase. 2, Usa. 3, Niyodo. 4, Kôchi. 5, Takiham. 6, Takuma.  
7, Hiroshima. 8, Owase. 9, Nagashima. 10, Lake Biwa. 11, Kanazawa.  
12, Wajima. 13, Takaya. 14, Takoshima. 15, Kugenuma. 16, Tokyo.  
17, Katsuura. 18, Taito. 19, Chôshi. 20, Ôta. 21, Lake Chûzenji.

vegetations were investigated in a waste salt-farm at Takiham (5), Ehime Prefecture, in the salt marsh and at the river-beach at Takuma (6), Kagawa Prefecture and on the seashore of Nagashima (9) and Owase (8), Mie Prefecture and on the seashore of Obase (1), Fukuoka Prefecture.

The osmotic values and NaCl contents of the strand dune plants were determined in *Carex Kobomugi* Ohwi, *Carex pumila* Thunb., *Ischaemum antheophoroides* Miq. var. *eristachyum* Honda, *Tournefortia sibirica* L., *Glehnia littoralis* Fr. Schm., *Lathyrus japonicus* Willd. and *Pinus Thunbergii* Parl., which were collected on the seashore of Kugenuma (15), Kanagawa Prefecture. The osmotic value and NaCl content were also measured in halophytes, such as *Salicornia herbacea* L., *Statice japonica* S. et Z., *Suaeda maritima* Dum. and *Suaeda japonica* Makino. These halophytes were collected at the places where soil conditions were investigated.

Experiments as to the effect of salt were carried out for the germination and growth of *Calystegia Soldanella* R. Br. and *Lathyrus japonicus* (strand plants), and *Suaeda japonica* (halophyte), and for the growth of seedlings of *Statice japonica* (halophyte).

## METHODS

After several fine days from a rain, soils were sampled at certain depths from the surface under the conditions ideal for the measurements of soil character as far as possible.

The pH value was determined by the colorimetric method with the solution which was prepared by shaking the fresh soil for 1 hour in distilled water 5 times the quantity of the soil sample. To determine the salt content of the soil, air-dried soil passed through a sieve of 0.5 mm. mesh was shaken with 5 times the quantity of distilled water for 1 hour, and chlorine ions were titrated with silver nitrate solution and listed as sodium chloride in the tables.

For the determination of the osmotic value, leaves were packed in glass vessels with cork stoppers and aluminium covers, and killed by boiling in water bath for 30 minutes [42]. Then the sap was expressed with a laboratory press and the osmotic value was determined by the cryoscopic method.

Chlorine ions in the expressed saps were determined by Steiner's method (27).

In order to test the effect of the concentration of salts or sea water on the germination and the growth, Brenner's artificial sea water with the following constitution was used (cf. Montfort and Brandrup [18]).

NaCl	20.2 g.	NaBr	0.08 g.
KCl	0.7 g.	KI	0.0002 g.
MgCl <sub>2</sub> ·6H <sub>2</sub> O	5.2 g.	KH <sub>2</sub> PO <sub>4</sub>	0.2 g.
MgSO <sub>4</sub> ·7H <sub>2</sub> O	3.1 g.	tap water	1000 g.
CaCl <sub>2</sub> ·6H <sub>2</sub> O	1.8 g.		

## RESULTS

## I. Soil conditions

## 1. Soil conditions of the strand dune vegetations

## A. The seashore of Taito, Chiba Prefecture

Soil conditions were investigated on the sandy seashore of Taito (18 in Fig. 1). Throughout this sandy seashore, *Wedelia prostrata* Hemsl., *Carex Kobomugi*, *Carex pumila*, *Tournefortia sibirica*, *Calystegia Soldanella*, *Ischaemum anthephoroides* var. *eriostachyum*, *Syntherisma sanguinalis* Dulac var. *ciliaris* Honda, and *Zoysia macrostachya* Fr. et Sav. were found abundant near the tide line. Besides them, *Lactuca repens* Maxim., *Vitex rotundifolia* L. f., *Artemisia capillaris* Thunb., *Orobanche ammophila* C. A. Mey., *Lathyrus japonicus*, *Linaria japonica* Miq., *Oenothera odorata* Jacq., *Fimbristylis sericea* R. Br., *Portulaca oleracea* L. and *Chenopodium acuminatum* Fr. et Sav. were found a little inland from the tide line, but not so abundant.

The results are shown in Tables 1, 2, 3 and 4. Soils were collected at a depth of 5 cm. from the surface if not otherwise specified. The salt content is expressed in percentage to the dry weight of the soil.

Salt contents and pH values of bare soils collected at the tide line were as follows: pH 8.8, NaCl content 0.383%, and Na<sub>2</sub>SO<sub>4</sub> content 0.060%. It was clear

TABLE 1

NaCl content and pH values of the soils collected in the plant communities  
and in bare soils about 10 metres inland from the tide line.  
On the seashore of Taito, Chiba Prefecture (Fig. 1-18)

Soils collected in the communities			Bare soils	
pH	NaCl content (%)	Vegetation	pH	NaCl content (%)
8.4	0.004	<i>Carex Kobomugi</i> , <i>Calystegia Soldanella</i> , <i>Syntherisma sanguinalis</i> var. <i>ciliaris</i>	9.2	0.072
8.2	0.003	<i>Wedelia prostrata</i>	9.0	0.057
8.8	0.003	<i>Zoysia macrostachya</i>	9.0	0.063
8.6	0.003	<i>Carex Kobomugi</i> , <i>Calystegia Soldanella</i>	9.0	0.063
8.6	0.004	<i>Wedelia prostrata</i>	8.8	0.070
8.8	0.003	<i>Carex Kobomugi</i> , <i>Carex pumila</i>	8.8	0.054
8.4	0.003	<i>Wedelia prostrata</i>	9.0	0.044
8.8	0.003	<i>Carex Kobomugi</i> , <i>Carex pumila</i>	8.8	0.020
8.8	0.004	<i>Carex Kobomugi</i> , <i>Carex pumila</i>	8.8	0.004
8.8	0.003	<i>Carex Kobomugi</i> , <i>Carex pumila</i>	8.8	0.020

TABLE 2

Salt content and pH values of the soils collected very close to the tide line.  
On the seashore of Taito, Chiba Prefecture (Fig. 1-18)

pH	NaCl content (%)	Na <sub>2</sub> SO <sub>4</sub> content (%)	Height	Vegetation
8.4	0.099	0.015	0.5 metre	<i>Carex Kobomugi</i>
8.8	0.006	trace	1 metre	<i>Carex Kobomugi</i> , <i>Wedelia prostrata</i>
8.6	0.004	trace	2 metres	<i>Carex Kobomugi</i> , <i>Wedelia prostrata</i>

that these high salt contents were due to the sea water, of which pH value, NaCl and Na<sub>2</sub>SO<sub>4</sub> contents were 8.4, 2.630% and 0.34%, respectively.

Table 1 shows the results of the investigation conducted in ten foremost communities about 10 metres inland from the tide line and in the bare soils just close to them on the sea side. The soils which were covered with vegetation contained a very small amount of NaCl (0.003–0.004%); on the other hand, the bare soils had NaCl about 10–20 times as much. Differences in NaCl content were perhaps caused by the fact that the communities developed on the slope sides about 1 metre higher than the bare soils. The bare soils were slightly more alkaline than the soils covered with vegetation. The presence of plants might make the soils less alkaline. Immediately after a storm, the soils covered with vegetation showed 0.022–0.057% NaCl content, and it was considerable that the NaCl content of these soils increased temporarily by the storm.

The results found in the foremost community very close to the tide line are summarized in Table 2. Soils at the height of 50 cm. above the tide line, being sprayed at times by the sea water at high tide, contained a very large amount



TABLE 3

NaCl content and pH values of the soils collected in the plant communities about 20-40 metres inland from the tide line.

On the seashore of Taito, Chiba Prefecture (Fig. 1-18)

pH	NaCl content (%)	Vegetation
8.6	trace	<i>Vitex rotundifolia</i> , <i>Calystegia Soldanella</i>
8.2	trace	<i>Wedelia prostrata</i> , <i>Calystegia Soldanella</i> , <i>Zoysia macrostachya</i>
8.8	0.003	<i>Wedelia prostrata</i>
8.8	trace	<i>Carex Kobomugi</i> , <i>Carex pumila</i>
8.8	0.003	<i>Wedelia prostrata</i>
8.6	0.003	<i>Vitex rotundifolia</i>
8.2	0.003	<i>Wedelia prostrata</i> , <i>Calystegia Soldanella</i> , <i>Artemisia capillaris</i> , <i>Orobancha ammophila</i>
8.6	0.003	<i>Calystegia Soldanella</i> , <i>Zoysia macrostachya</i>
8.4	0.003	<i>Calystegia Soldanella</i> , <i>Zoysia macrostachya</i>
8.4	0.003	<i>Tournefortia sibirica</i>

TABLE 4

NaCl content and pH values of the soils collected in the plant communities about 70-100 metres inland from the tide line.

On the seashore of Taito, Chiba Prefecture (Fig. 1-18)

pH	NaCl content (%)	Vegetation
7.4	trace	<i>Carex Kobomugi</i>
7.4	trace	<i>Artemisia capillaris</i>
7.4	0.003	<i>Carex Kobomugi</i>

of NaCl, i. e. 0.099%. Such a remarkably high level was never seen in any other soils covered with vegetation on this seashore. It is interesting that *Carex Kobomugi* could tolerate such a high salinity of the soil and occasional spray of the sea water.

Table 3 shows the results of the investigation conducted in communities about 20-40 metres inland from the tide line, and Table 4, those of about 70-100 metres inland. As seen in these tables, NaCl contents decreased slightly with increasing the distance from the tide line and the soils were a little less alkaline at about 70-100 metres inland from the tide line.

In some spots NaCl contents of the soils were determined at three different depths (5, 15 and 30 cm.), and there was no difference in the vertical direction.

B. The seashore of Usa and the sandy strand at the river-mouth of the Niyodo River, Kôchi Prefecture

The investigations were conducted on the sandy seashore of Usa (2 in Fig. 1) and on the sandy strand at the river-mouth of the Niyodo River (3).

TABLE 5

NaCl content and pH values of the soils collected on the seashore of Usa,  
Kôchi Prefecture (Fig. 1-2)

Station	Approximate distance from the tide line	pH	NaCl content (%)	Vegetation
1	tide line	7.8	0.185	Bare soils
	20 metres	7.8	0.003	Bare soils
	30 "	7.6	0.003	Bare soils
	35 "	6.8	trace	<i>Vitex rotundifolia</i>
	45 "	6.8	trace	<i>Calystegia Soldanella</i> , <i>Syntherisma sanguinalis</i> var. <i>ciliaris</i>
2	tide line	7.2	0.157	Bare soils
	15 metres	7.2	0.003	Bare soils
	20 "	7.2	trace	Bare soils
	50 "	6.4	trace	<i>Vitex rotundifolia</i> , <i>Syntherisma sanguinalis</i> var. <i>ciliaris</i>

TABLE 6

NaCl content and pH values of the soils collected on the sandy strand at the  
river-mouth of the Niyodo River, Kôchi Prefecture (Fig. 1-3)

Station	Approximate distance from the tide line	pH	NaCl content (%)	Vegetation
1	60 metres	7.2	0.003	Bare soils
	65 "	6.6	0.003	<i>Carex Kobomugi</i> , <i>Glehnia littoralis</i>
	80 "	6.6	0.003	<i>Carex Kobomugi</i>
2	55 "	7.2	0.003	Bare soils
	60 "	7.0	trace	<i>Carex Kobomugi</i>
	70 "	6.6	trace	<i>Carex Kobomugi</i>
	85 "	6.4	trace	<i>Carex Kobomugi</i>
3	50 "	7.0	trace	Bare soils
	65 "	6.4	trace	<i>Glehnia littoralis</i>
	85 "	6.2	trace	<i>Carex Kobomugi</i> , <i>Glehnia littoralis</i>
4	60 "	7.2	0.003	Bare soils
	65 "	6.4	trace	<i>Carex Kobomugi</i> , <i>Lactuca repens</i> , <i>Glehnia littoralis</i>
	85 "	6.6	trace	<i>Carex Kobomugi</i> , <i>Glehnia littoralis</i>

On the seashore of Usa about 30 metres inland from the tide line, communities of *Vitex rotundifolia*, *Calystegia Soldanella* and *Syntherisma sanguinalis* var. *ciliaris* were found on the sandy hillocks about 1 metre high. As seen in Table 5, soils near the tide line contained much NaCl (0.157–0.185%), but bare soils a little inland from the tide line had a low NaCl content and soils covered with vegetation contained only a trace of NaCl.

Table 6 shows the results obtained on the long sandy strand at the river-mouth of the Niyodo River. Here a wide bare strand about 60 metres wide extended along the tide line, and inside the bare strand, communities of *Carex Kobomugi*, *Glehnia littoralis* and *Lactuca repens* were found on 1–2 metre level. The soils except for those collected near the tide line had a low NaCl content and most of the soils covered with vegetation contained only traces of NaCl. The pH values were in the range of 6.2–7.8, the soils covered with vegetation being slightly acid.

As compared with the results obtained at the seashore of Taito, the soils investigated at Usa and the Niyodo River had a similar NaCl content but were slightly less alkaline than those at Taito.

#### C. The seashore in the vicinity of Kanazawa, Ishikawa Prefecture

In the vicinity of Kanazawa (11 in Fig. 1), there develop long and wide sandy strands and dunes facing the Japan Sea and at certain places their widths reach about 1,000 metres. At some of such stations the soil conditions were investigated.

On these strands and dunes, *Carex Kobomugi* and *Wedelia prostrata* grew abundantly near the tide line. Besides them, *Vitex rotundifolia*, *Linaria japonica*, *Zoysia macrostachya*, *Ischaemum antheplioroides* var. *eristachyum*, *Glehnia littoralis*, *Artemisia capillaris* and *Aster Asa-Grayi* Makino were found mostly a little inland from the tide line. There grew also *Carex pumila* here and there but not so abundantly.

The NaCl contents and pH values of bare soils near the tide line were 0.120–0.125% and 8.0, respectively.

Soil conditions were investigated in the foremost communities about 20 metres inland from the tide line and the results are shown in Table 7. One of the investigated stations indicated an exceptionally high NaCl content (0.056%), as was experienced similarly on the seashore of Taito.

TABLE 7

NaCl content and pH values of the soils collected in the plant communities about 20 metres inland from the tide line, in the vicinity of Kanazawa (Fig. 1-11)

pH	NaCl content (%)	Vegetation
7.2	0.056	<i>Carex Kobomugi</i>
7.0	0.008	<i>Lactuca repens</i> , <i>Calystegia Soldanella</i>

The investigations were conducted in five foremost communities about 40-70 metres inland from the tide line and also in the bare soils just close to them (Table 8). In the pH values there was no clear difference among these stations. As in the case of the seashore of Taito, the soils which were covered with vegetation indicated lower NaCl contents (0.003-0.005%) and the bare soils contained about 6-10 times the quantity of NaCl as the soils covered with vegetation.

About 30-60 metres inland from the tide line, soils were nearly neutral and contained mostly about 0.003-0.004% NaCl as seen in Table 9. Tables 10 and 11 show the results obtained at the stations about 150 metres and about 300-700 metres inland from the tide line. NaCl contents decreased with the distance, and soil reactions turned slightly acid.

TABLE 8  
NaCl content and pH values of the soils collected in the plant communities and in bare soils about 40-70 metres inland from the tide line, in the vicinity of Kanazawa (Fig. 1-11)

Soils collected in the communities			Bare soils	
pH	NaCl content (%)	Vegetation	pH	NaCl content (%)
8.2	0.003	<i>Carex Kobomugi</i> , <i>Calystegia Soldanella</i>	8.2	0.035
7.4	0.005	<i>Carex Kobomugi</i>	7.2	0.032
7.6	0.003	<i>Carex Kobomugi</i>	7.6	0.020
8.0	0.003	<i>Carex Kobomugi</i>	8.2	0.018
7.6	0.003	<i>Calystegia Soldanella</i>	7.6	0.020

TABLE 9  
NaCl content and pH values of the soils collected in the plant communities about 30-60 metres inland from the tide line, in the vicinity of Kanazawa (Fig. 1-11)

pH	NaCl content (%)	Vegetation
7.2	0.004	<i>Carex Kobomugi</i> , <i>Linaria japonica</i>
7.0	0.004	<i>Carex Kobomugi</i>
7.0	0.004	<i>Carex Kobomugi</i>
7.2	0.006	<i>Carex Kobomugi</i>
7.0	trace	<i>Carex Kobomugi</i> , <i>Tournefortia sibirica</i> , <i>Linaria japonica</i>
7.0	0.003	<i>Carex Kobomugi</i> , <i>Tournefortia sibirica</i>
7.0	0.003	<i>Carex Kobomugi</i> , <i>Lactuca repens</i>
6.6	0.004	<i>Vitex rotundifolia</i>
6.4	0.003	<i>Carex Kobomugi</i>
6.6	0.003	<i>Tournefortia sibirica</i>
7.0	0.003	<i>Carex Kobomugi</i>
7.0	0.003	<i>Carex Kobomugi</i>



TABLE 10

NaCl content and pH values of the soils collected in the plant communities about 150 metres inland from the tide line, in the vicinity of Kanazawa (Fig. 1-11)

pH	NaCl content (%)	Vegetation
6.6	0.003	<i>Lactuca repens</i>
6.4	0.003	<i>Carex Kobomugi</i>
6.4	0.004	<i>Ischaemum antheophoroides</i> var. <i>eristachyum</i>

TABLE 11

NaCl content and pH values of the soils collected in the plant communities far away from the tide line, in the vicinity of Kanazawa (Fig. 1-11)

Approximate distance from the tide line	pH	NaCl content (%)	Vegetation
300 metres	6.4	trace	<i>Ischaemum antheophoroides</i> var. <i>eristachyum</i> , <i>Artemisia capillaris</i> , <i>Calystegia Soldanella</i>
300 "	6.4	trace	<i>Ischaemum antheophoroides</i> var. <i>eristachyum</i> , <i>Artemisia capillaris</i> , <i>Calystegia Soldanella</i>
300 "	6.4	trace	<i>Carex Kobomugi</i> , <i>Linaria japonica</i> , <i>Artemisia capillaris</i>
500 "	6.2	trace	<i>Carex Kobomugi</i> , <i>Glehnia littoralis</i>
500 "	6.4	trace	<i>Ischaemum antheophoroides</i> var. <i>eristachyum</i>
700 "	6.2	trace	<i>Ischaemum antheophoroides</i> var. <i>eristachyum</i> , <i>Carex Kobomugi</i>
700 "	6.2	trace	<i>Robinia pseudo-Acacia</i>

#### D. The seashores in the Noto Peninsula, Ishikawa Prefecture

The investigations were conducted on the sandy seashores of Takoshima (14 in Fig. 1) and Takaya (13) in the Noto Peninsula, and the results obtained are summarized respectively in Tables 12 and 13.

On the seashore of Takoshima, *Salsola Komarovi* Iljin was found.

As compared with the results obtained in the vicinity of Kanazawa, here the soils were more alkaline, but NaCl contents were similar in both strands.

As will be seen from the foregoing results, most of the soils which were covered with vegetation had a very low NaCl content (0.003-0.004% or sometimes only in traces) and NaCl content decreased with the distance from the sea. On the seashore of Taito and likewise on the seashore in the vicinity of Kanazawa, however, the soils in the communities of *Carex Kobomugi*, which lay very close to the tide line contained remarkably much NaCl, 0.099% at the former station and 0.056% at the latter: *Carex Kobomugi* can grow on the soils of such a high NaCl content, with its special characteristics for tolerating high salinity of the soil.

TABLE 12

NaCl content and pH values of the soils collected on the seashore of Takoshima, Ishikawa Prefecture (Fig. 1-14)

Approximate distance from the tide line	pH	NaCl content (%)	Vegetation
tide line	8.2	0.008	Bare soils
30 metres	8.2	0.004	<i>Ischaemum anthephoroides</i> var. <i>eristachyum</i> , <i>Artemisia capillaris</i>
30 "	8.2	0.004	<i>Ischaemum anthephoroides</i> var. <i>eristachyum</i> , <i>Carex Kobomugi</i>
30 "	8.2	0.004	<i>Ischaemum anthephoroides</i> var. <i>eristachyum</i> , <i>Carex Kobomugi</i>
150 "	8.0	0.003	<i>Salsola Komarovi</i>
150 "	8.0	trace	<i>Ischaemum anthephoroides</i> var. <i>eristachyum</i> , <i>Carex pumila</i>

TABLE 13

NaCl content and pH values of the soils collected on the seashore of Takaya, Ishikawa Prefecture (Fig. 1-13)

Approximate distance from the tide line	pH	NaCl content (%)	Vegetation
30 metres	8.2	trace	<i>Tournefortia sibirica</i> , <i>Calystegia Soldanella</i> , <i>Lactuca repens</i> , <i>Carex pumila</i>
30 "	8.2	trace	<i>Tournefortia sibirica</i> , <i>Calystegia Soldanella</i> , <i>Lactuca repens</i>

Kearney [14] and also Weaver and Clements [46] have already suggested that the precipitation is one of the most important factors influencing the salt content of the soil. The annual precipitation of the main stations is as follows: 1,664 mm. at Chôshi (19 in Fig. 1), 2,526 mm. at Kanazawa (11), and 2,185 mm. at Wajima (12). According to Kearney [14], the precipitation and salt content of soils are at Norfolk 1,302 mm. and 0.003-0.02%, and at Los Angeles 393 mm. and 0.02-0.15%, respectively. Therefore, it seems to be decisive that the soils of the seashore investigated by the writer, being washed with high precipitation, have a lower salt content as compared with the soils at Los Angeles with low precipitation.

The soils covered with vegetation were alkaline on the seashore of Taito and in the Noto Peninsula (pH 7.4-8.8), but on the seashores in Kôchi Prefecture and in the vicinity of Kanazawa, soils were nearly neutral or slightly acid (pH 6.2-8.2). Salisbury observed in his studies on the dunes in England that the pH values of the soils were 5.5-7.6 [21] and 5.5-8.2 [22]. On the investigated strands of Japan, soil reactions turned gradually from alkaline to acid with increasing distance from the sea, as already reported by Salisbury [21, 22] and Kurz [17] in England and America, respectively.

On the sandy strands which were investigated, *Carex Kobomugi* was found ubiquitous and abundant. Besides this species, *Wedelia prostrata*, *Calystegia Soldanella*, *Tournefortia sibirica*, *Ischaemum antheophoroides* var. *eristachyum*, *Lactuca repens*, *Zoysia macrostachya* and *Vitex rotundifolia* were found in common on the seashore of Taito as well as in the vicinity of Kanazawa. Accordingly it may be considered that the main constituent species of the strand dune vegetations are common to both the Pacific coast and the Japan Sea coast.

## 2. Soil conditions of the halophytic vegetations

Hydrogen-ion concentrations, water contents and NaCl contents were investigated in the soils where halophytes, *Salicornia herbacea*, *Statice japonica*, *Suaeda maritima* and *S. japonica*, were growing. Soils were collected at the depth of 5 cm. The water content of soil was represented in percentage to the fresh weight of soil and the NaCl content was expressed in percentage as well to the dry weight of soil as to the water amount contained in the soil.

TABLE 14  
Soil conditions investigated in the communities of *Salicornia herbacea*

pH	Soil water content (%)	NaCl content based on the dry weight of soil (%)	NaCl content based on the water content of soil (%)	Locality
6.0	23.4	0.82	3.5	salt marsh at Takuma, Kagawa Prefecture (Fig. 1-6)
6.2	24.2	0.47	1.9	
7.2	21.2	0.41	1.9	
7.2	27.7	0.18	0.6	waste salt-farm at Takihama, Ehime Prefecture (Fig. 1-5)
7.6	17.6	1.05	5.9	
8.4	21.2	1.75	8.2	
8.6	19.0	1.23	6.4	
7.0	36.9	0.12	0.3	hillocks in the waste salt-farm at Takihama, Ehime Prefecture (Fig. 1-5)
6.0	16.2	0.58	3.5	
8.6	20.4	0.88	4.3	
6.8	52.0	0.88	1.6	salt marshes in the waste salt-farm at Takihama, Ehime Prefecture (Fig. 1-5)
6.6	21.5	0.58	2.6	
7.4	35.1	0.64	1.8	
6.6	24.6	0.53	2.1	
7.0	45.9	1.11	2.4	
6.0	19.7	1.17	5.9	
6.4	25.3	1.17	4.6	
7.2	36.0	1.58	4.3	
7.6	25.0	0.88	3.5	
8.4	23.4	0.76	3.2	
7.8	48.5	1.46	3.0	

A. Communities of *Salicornia herbacea*

Soils were collected in the salt marsh at Takuma (6 in Fig. 1), Kagawa Prefecture, and in the waste salt-farm at Takihamma (5), Ehime Prefecture. In the latter, *Salicornia herbacea* grew on the ground, on hillocks and at the margin of the salt marsh. At the margin of the salt marsh the halophyte grew generally thicker than on the other places. The results concerned with pH, water content and NaCl content are summarized in Table 14.

B. Communities of *Statice japonica*

Soils were collected at the river-beach of the Takase River at Takuma, in the waste salt-farm at Takihamma, and at the seashores of Nagashima and Owase, Mie Prefecture, and Obase, Fukuoka Prefecture (9, 8 and 1 in Fig. 1, respectively), and the results are presented in Table 15.

TABLE 15  
Soil conditions investigated in the communities of *Statice japonica*

pH	Soil water content (%)	NaCl content based on the dry weight of soil (%)	NaCl content based on the water content of soil (%)	Locality
6.4	12.6	0.35	2.7	river-beach of the Takase River at Takuma, Kagawa Prefecture (Fig. 1-6)
7.0	29.8	0.58	1.9	
6.0	16.2	0.58	3.5	hillocks in the waste salt-farm at Takihamma, Ehime Prefecture (Fig. 1-5)
6.2	22.6	0.29	1.2	
7.6	22.6	0.11	0.4	
7.8	23.4	0.58	2.4	
7.4	15.2	1.35	8.8	seashore of Nagashima, Mie Prefecture (Fig. 1-9)
7.4	22.5	0.82	3.6	
6.8	22.9	1.23	5.3	
7.0	31.0	1.05	3.3	
6.6	18.4	0.29	1.5	seashore of Owase, Mie Prefecture (Fig. 1-8)
7.8		0.46		seashore of Obase, Fukuoka Prefecture (Fig. 1-1)
7.8		0.43		

TABLE 16  
Soil conditions investigated in the communities of *Suaeda maritima*

pH	Soil water content (%)	NaCl content based on the dry weight of soil (%)	NaCl content based on the water content of soil (%)	Locality
7.2	22.6	0.82	3.6	waste salt-farm at Takihamma, Ehime Prefecture (Fig. 1-5)
6.8	16.9	0.76	4.4	
7.8	23.4	0.58	2.4	



C. Communities of *Suaeda maritima*

Soils were collected in the waste salt-farm at Takihamma and the results are given in Table 16.

D. Communities of *Suaeda japonica*

Soils were collected at the river-beach of the Takase River at Takuma and at the seashore of Obase. The results obtained are shown in Table 17.

TABLE 17  
Soil conditions investigated in the communities of *Suaeda japonica*

pH	Soil water content (%)	NaCl content based on the dry weight of soil (%)	NaCl content based on the water content of soil (%)	Locality
6.4	12.6	0.35	2.7	river-beach of the Takase River at Takuma, Kagawa Prefecture (Fig. 1-6)
6.2	20.8	0.82	3.9	
7.4		0.82		seashore of Obase, Fukuoka Prefecture (Fig. 1-1)
7.4		0.64		
7.2		0.51		
7.8		0.77		
7.6		0.97		
7.6		0.69		

TABLE 18  
Variations in soil conditions at different depths or heights.  
In the waste salt-farm at Takihamma, Ehime Prefecture (Fig. 1-5)

Station	pH	Soil water content (%)	NaCl content based on the dry weight of soil (%)	NaCl content based on water content of soil (%)	Depth or height
1	8.4	21.2	1.75	8.2	at the depth of 5 cm.
	7.4	21.9	0.94	4.2	at the depth of 15 cm.
2	8.6	19.0	1.23	6.4	at the depth of 5 cm.
	7.4	24.2	0.82	3.3	at the depth of 15 cm.
3	7.6	17.6	1.05	5.9	at the depth of 5 cm.
	6.6	21.2	0.41	1.9	at the depth of 15 cm.
4	7.8	48.5	1.46	3.0	on the ground level
	7.6	22.6	0.11	0.4	on the hillock 0.6 metre high
5	7.8	23.4	0.58	2.4	on the ground level
	7.2	27.1	0.02	0.1	on the hillock 0.6 metre high
6	8.4	13.6	0.41	3.0	on the ground level
	8.2	5.2	0.14	2.6	on the hillock 1 metre high

Soil conditions at different depth and elevations at the same place were investigated in the waste salt-farm at Takihamma. As seen in Table 18, the soils from deeper layer indicated the less alkaline reaction, lower NaCl content and higher water content, and the high-level soils revealed lower NaCl content than the ground-surface soils.

As will be seen from the foregoing results, the soils collected in *Salicornia herbacea* communities had a higher water content than those collected in the communities of the other three halophytic species. It was observed that *Salicornia herbacea* grew thicker on wet soils at the margin of the salt marsh than on the ordinary ground and on the hillock. The pH values fell in the range between 6.0 and 8.6, with considerable local variations. In NaCl contents of the soils also considerable local variations were found, and the soils collected in the *Salicornia herbacea* communities had somewhat higher NaCl contents than the soils of the communities of the other three species. It was reported also by the foreign investigators that the soils collected in *Salicornia* communities were higher in NaCl content than the soils collected in *Suaeda* communities. Hatanaka [12] recently reported on the NaCl content of soils of halophytic communities at Sone, near Obase (1 in Fig. 1), and found that the soils from the *Suaeda japonica* communities were slightly superior to those from the *Statice japonica* communities in NaCl content. The NaCl amounts found by the present writer of the soils collected in the *Salicornia* and *Suaeda* communities were similar to those reported by the foreign investigators.

Comparing the soil conditions of the strand dune vegetations with those of halophytic ones, we can find an important fact that the soils collected in the former vegetations have a remarkably lower NaCl content than those collected in the halophytic vegetations. Attention must be paid to this fact. We should regard this remarkable difference in NaCl content of the soils in these two vegetations as the first important criterion to make a clear distinction in the halophilic characteristics between the strand dune plants and the halophytes.

## II. Osmotic values and NaCl content of plant saps

### 1. Strand dune plants

Annual fluctuations in osmotic values and NaCl contents of leaf saps were determined in *Carex Kobomugi*, *Tournefortia sibirica*, *Carex pumila*, *Ischaemum antheophoroides* var. *eristachyum*, *Glehnia littoralis*, *Lathyrus japonicus* and *Pinus Thunbergii*; these grew on the sandy seashore of Kugenuma (15 in Fig. 1), Kanagawa Prefecture. The first and second species were collected on the dunes and the other species, in a wood of *Pinus Thunbergii* close to the dunes. Soil conditions were on the dunes pH 7.6-8.6 and NaCl content 0.003-0.004%, while in the pine wood they were pH 7.0-7.4 and NaCl content in traces, or sometimes up to 0.003%.

Determinations were carried on from April to December, and the samples were collected under the conditions identical as far as possible, i. e. between

TABLE 19  
Annual fluctuations in the osmotic value, NaCl content of leal saps and water content of the leaf.  
I. Kugenuma, Kanagawa Prefecture (Fig. 1-15)

Date	Carex Kobomugi					Carex Kobomugi					Tournefortia sibirica					Carex pumila				
	Osmotic value (atm.)	NaCl content of leaf sap (atm.)	O <sub>NaCl</sub> /Osm (%)	Leaf water content (%)	Soil water content (%)	Osmotic value (atm.)	NaCl content of leaf sap (atm.)	O <sub>NaCl</sub> /Osm (%)	Leaf water content (%)	Soil water content (%)	Osmotic value (atm.)	NaCl content of leaf sap (atm.)	O <sub>NaCl</sub> /Osm (%)	Leaf water content (%)	Soil water content (%)	Osmotic value (atm.)	NaCl content of leaf saps (atm.)	O <sub>NaCl</sub> /Osm (%)	Leaf water content (%)	Soil water content (%)
Apr. 24	12.2	3.8	31	76	7.0	13.3	6.1	46	72	7.5						16.8	4.7	28	57	7.0
May 17	13.2	4.7	36	70	4.0	14.0	6.8	49	73	4.5						14.8	4.4	30	56	7.3
June 14	12.8	5.8	45	70	5.5	13.1	6.1	47	71		3.4	34	81			13.6	3.9	29	57	8.0
June 28	12.3	4.4	36	73	6.5	12.6	4.7	37	75	6.3	3.1	36	85			17.0	5.1	30	65	3.5
July 7	13.5	5.4	40	72	4.3	14.7	6.5	44	73	5.0	3.9	37	84			16.2	5.1	31	59	6.3
July 27	14.9	7.1	48	65	4.8	14.8	7.1	48	66	4.8	4.0	34	78			15.2	6.1	40	68	7.8
Aug. 3	15.0	8.9	59	75	5.8	14.4	7.1	49	75	5.3	6.8	51	83			18.4	7.1	39		
Sept. 12	17.9	9.9	55	73		15.3	7.1	46	73		5.7	42	85							
Sept. 30	13.9	7.1	51	75		13.5	6.8	50	76											
Oct. 10	15.0	8.2	55	68		15.4	6.1	40	73	14.8	8.1	55	82			17.9	7.1	40	59	7.3
Oct. 24	15.5	7.8	50	68	7.0	15.2	7.6	50	70	6.0						16.7	6.8	41	58	
Nov. 7	18.0	9.2	51	71		18.8	9.2	49	71		9.9	64	85			16.1	6.5	40	57	
Nov. 25	20.8	9.2	44	70		19.2	10.2	53	67							17.9	6.1	34	47	
Dec. 9				46					26							19.6	8.5	43	42	
Average	15.0	7.0	46	69	5.6	14.9	7.0	47	69	5.6	12.2	5.6	44	83	16.7	6.0	35	57	6.7	

TABLE 20  
Annual fluctuations in the osmotic value, NaCl content of leaf saps and water content of the leaf.  
II. Kugenuma, Kanagawa Prefecture (Fig. 1-15)

Date	<i>Ischaemum antheophoroides</i> var. <i>eriostachyum</i>				<i>Glehnia littoralis</i>				<i>Lathyrus japonicus</i>				<i>Pinus Thunbergii</i>			
	Osmotic value (atm.)	NaCl content of leaf sap (atm.)	$O_{NaCl}/O_{sm}$ (%)	Leaf water content (%)	Osmotic value (atm.)	NaCl content of leaf sap (atm.)	$O_{NaCl}/O_{sm}$ (%)	Leaf water content (%)	Osmotic value (atm.)	NaCl content of leaf sap (atm.)	$O_{NaCl}/O_{sm}$ (%)	Leaf water content (%)	Osmotic value (atm.)	NaCl content of leaf sap (atm.)	$O_{NaCl}/O_{sm}$ (%)	Leaf water content (%)
Apr. 24																
May 17																
June 14					11.7	4.4	38	77	11.8	2.0	17	81	19.2	1.0	5	48
June 28									11.0	1.8	16	80				
July 7					12.5	5.8	46	80	12.2	2.7	22	63	16.6	0.7	4	52
July 27	8.9	2.6	29	51					10.1	1.4	14	73				
Aug. 3					12.5	6.8	54	80	11.5	2.0	17	76	18.4	1.0	5	64
Sept. 12	8.7	3.7	42	65	14.6	8.8	60	80	10.7	2.0	19	82				
Sept. 30	8.9	3.5	39	68					9.9	2.6	26	83	17.4	0.7	4	60
Oct. 10					12.8	6.6	52	79	10.5	2.4	23	80				
Oct. 24	10.1	3.7	37	44					10.2	2.4	24	85	17.7	0.7	4	60
Nov. 7	10.1	3.7	37	63					12.8	4.8	38	80	18.6	1.4	8	59
Nov. 25	17.2	5.8	34	47					14.8	4.8	32	78	21.5	1.4	7	58
Dec. 9				10												
Average	10.7	3.8	36	50	12.8	6.5	50	79	11.4	2.6	23	78	18.5	1.0	5	57



noon and 2 p.m. after several fine days. The results are summarized in Tables 19 and 20, and illustrated in Figs. 2 and 3. Osmotic values of leaf saps were indicated in atm. and NaCl contained in leaf saps was expressed with an estimation of its osmotic value in atm.

As seen in the tables, *Carex pumila* and *Carex Kobomugi* showed the highest osmotic values of the plants investigated except for *Pinus Thunbergii*; *Glehnia littoralis* and *Tournefortia sibirica* were somewhat lower in osmotic value, and *Lathyrus japonicus* and *Ischaemum antheplhoroides* var. *eristachyum* gave the lowest. Hori [13] cryoscopically determined the osmotic values of some strand dune plants of Japan and obtained a little higher values in *Tournefortia sibirica*, *Glehnia littoralis*, *Carex Kobomugi* and *Lathyrus japonicus* than those obtained by the writer. Takada [30-33] also reported cryoscopical determinations of the osmotic values in some Japanese strand plants and there were obtained a little higher values in *Glehnia littoralis* than those obtained by the writer. These may be due to the differences of the habitat conditions at the sampling

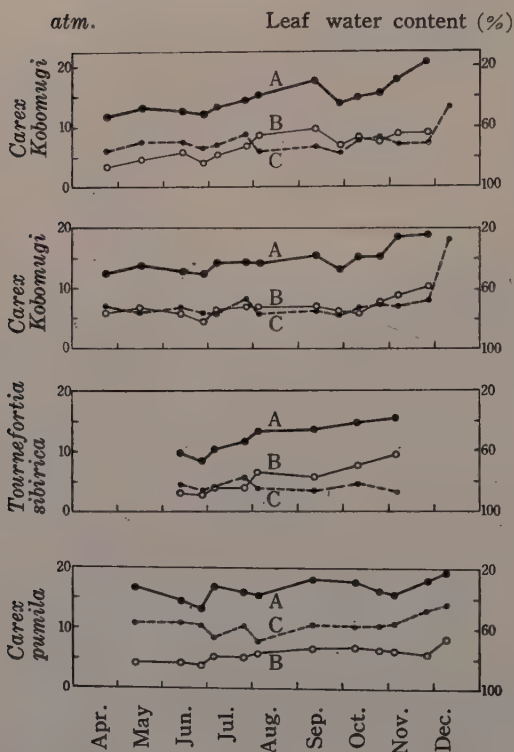


Fig. 2. Annual fluctuations in osmotic value, NaCl content of leaf saps and water content on a fresh weight basis of the leaf. A (thick line), osmotic value. B (fine, solid line), NaCl content (in atm.) of leaf saps. C (fine, broken line), water content of the leaf.

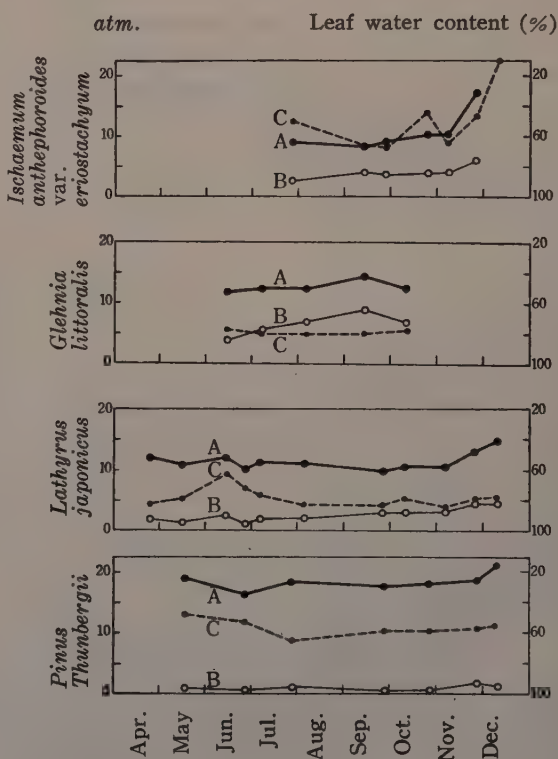


Fig. 3. Annual fluctuations in osmotic value, NaCl content of leaf saps and water content on a fresh weight basis of the leaf. A, B and C indicate the same in Fig. 2.

stations. Volk [41], Harris [11], Steiner [27] and Walter and Steiner [45] reported the osmotic values of strand and of dune plants determined cryoscopically. The writer could find in many of them similar values to those determined by himself, though the observed species were not the same. In the plants which had been transplanted in the Botanical Garden of the University of Tokyo, the writer obtained somewhat lower osmotic values, i. e. in *Carex Kobomugi* 11.2–13.0 atm. in May and in *Lathyrus japonicus* 9.0–10.0 atm. in May, than those determined in the same species naturally grown on the sandy seashore.

In the annual fluctuations of the osmotic values of the plant saps, several types were reported by Thren [34]. In the present investigation higher values were generally observed in summer and autumn than in spring, and markedly high values were seen in yellowing leaves in autumn and in winter.

Except for the case of *Pinus Thunbergii*, the osmotic values generally change according to the amounts of NaCl in plant saps (Figs. 2 and 3), and from this fact it may be suspected that NaCl in plant saps plays an important part in shifting the osmotic value. To investigate the role in the osmotic value of NaCl in plant saps in detail, the ratio of the estimated osmotic value caused by

TABLE 21

Osmotic value and NaCl content of expressed saps of *Salicornia herbacea* collected at Takihamma (Ehime Prefecture: Fig. 1-5) and Takuma (Kagawa Prefecture: Fig. 1-6)

Osmotic value (atm.)	NaCl content of expressed saps (atm.)	$O_{NaCl}/O_{sm}$ (%)	NaCl content of the soil		Locality	Month
			based on the dry weight of soil (%)	based on the water content of soil (%)		
21.0	17.0	81	0.18	0.6	waste salt-farm at Takihamma	Jan.
25.4	18.0	71	0.88	1.6		
40.3	37.0	92	0.82	3.5	salt marsh at Takuma	
42.8	38.5	90	0.47	1.9		
47.0	44.0	94	1.05	5.9	waste salt-farm at Takihamma	Aug.
45.6	41.5	91	0.58	3.5		
44.5	41.5	93	1.11	2.4		
46.7	43.0	92	1.17	5.9		
44.3	40.5	91	1.17	4.6		
62.1	60.0	97	0.41	1.9	salt marsh at Takuma	
57.2	54.0	94				
75.4	71.5	95	1.75	8.2	waste salt-farm at Takihamma	Nov.
70.2	66.0	94	1.23	6.4		
68.3	61.5	90	0.88	4.3		
63.3	58.5	92	0.88	3.5		
56.0	54.0	96	0.76	3.2		
58.1	55.5	96	1.46	3.0		

NaCl in plant saps to the total osmotic value determined by cryscopy was expressed in percentage, which was indicated in the tables as  $O_{NaCl}/Osm$  percentage. The annual mean values were as follows: 50% in *Glehnia littoralis*, 46% and 47% in *Carex Kobomugi*, 44% in *Tournefortia sibirica*, 36% in *Ischaemum antheophoroides* var. *eristachyum*, 35% in *Carex pumila*, and 23% in *Lathyrus japonicus*. Thus though slight differences were seen with species, it may be regarded that the  $O_{NaCl}/Osm$  percentage is, like the osmotic value, one of the standards representing halophilic features of the plants. Walter and Steiner [45] have reported that the  $O_{NaCl}/Osm$  percentages of nonhalophytes and of the plants which grew in NaCl-poor soils were generally less than 50%. In the present investigation the writer has found the similar values of annual mean  $O_{NaCl}/Osm$  percentage in plants collected on the sandy seashore where soils contained a very small amount of NaCl.

## 2. Halophytes

Halophytes, such as *Salicornia herbacea*, *Statice japonica*, *Suaeda maritima* and *Suaeda japonica*, were investigated in their osmotic values and NaCl contents of plant saps, which were expressed from leaves in the *Statice* and from the shoots of three other species. The results are summarized in Tables 21, 22, 23 and 24.

TABLE 22

Osmotic value and NaCl content of expressed saps of *Statice japonica*, collected at Nagashima and Owase Mie Prefecture (Fig. 1-9 and -8) Takuma, Kagawa Prefecture (Fig. 1-6) and Takiham, Ehime Prefecture (Fig. 1-5)

Osmotic value (atm.)	NaCl content of expressed saps (atm.)	$O_{NaCl}/O_{sm}$ (%)	NaCl content of the soil		Locality	Month
			based on the dry weight of soil (%)	based on the water content of soil (%)		
36.5	27.5	75	1.35	8.8	seashore of Nagashima	Jan.
31.8	21.0	66	0.82	3.6		
35.8	27.5	77	0.29	1.5	seashore of Owase	
30.7	23.0	74	0.35	2.7	river-beach of the Takase River at Takuma	Aug.
33.3	25.5	77	0.58	3.5	waste salt-farm at Takiham	
26.9	21.5	80	0.29	1.2		
26.6	16.5	62	0.58	1.9	river-beach of the Takase River at Takuma	Nov.
27.9	14.5	52	0.11	0.4	waste salt-farm at Takiham	
28.6	18.0	63	0.58	2.4		

TABLE 23

Osmotic value and NaCl content of expressed saps of *Suaeda maritima* collected in the waste salt-farm at Takiham, Ehime Prefecture (Fig. 1-5)

Osmotic value (atm.)	NaCl content of expressed saps (atm.)	$O_{NaCl}/Osm$ (%)	NaCl content of the soil		Month
			based on the dry weight of soil (%)	based on the water content of soil (%)	
32.2	25.5	79	0.82	3.6	Aug.
36.3	30.5	84	0.76	4.4	
34.3	26.0	76	0.58	2.4	Nov.

TABLE 24

Osmotic value and NaCl content of expressed saps of *Suaeda japonica* collected at the river-beach of the Takase River at Takuma, Kagawa Prefecture (Fig. 1-6), in August

Osmotic value (atm.)	NaCl content of expressed saps (atm.)	$O_{NaCl}/Osm$ (%)	NaCl content of the soil	
			based on the dry weight of soil (%)	based on the water content of soil (%)
30.0	24.0	78	0.35	2.7
30.3	21.5	71	0.82	3.9

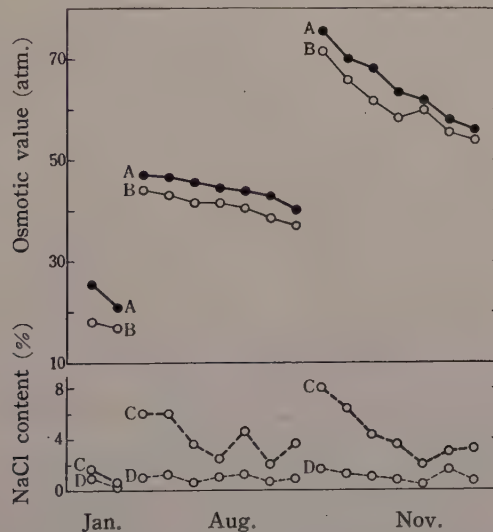


Fig. 4. *Salicornia herbacea*. Osmotic value and NaCl content of expressed saps, and NaCl content of the soil. A: osmotic value. B: NaCl content (in atm.) of expressed saps. C: NaCl content of the soil based on the water content of soil. D: NaCl content of the soil based on the dry weight of soil.



Investigations were conducted in *Salicornia herbacea*, in January in immature plants and in August and November in mature plants. Consequently lower osmotic values and NaCl contents were obtained in January. In the three other species, determinations were all conducted in mature plants. *Salicornia* was higher in osmotic value and  $O_{NaCl}/Osm$  percentage than the three other species.

It could not be clarified whether NaCl in the soils played a part in shifting the osmotic value and NaCl content of the plant saps, as conclusive determinations were difficult to be carried out. In several cases, however, *Salicornia herbacea* and *Statice japonica* raised their osmotic values and NaCl content clearly with the increases of NaCl amounts in the soils (Figs. 4 and 5). Arnold [2] and Schratz [24] observed the fact that *Salicornia* and *Suaeda* which grew in NaCl-rich soils generally contained much NaCl in them. Beadle, Whalley and Gibson [4], however, reported that in *Atriplex* plants, the percentage of chloride was approximately the same irrespective of the concentration of NaCl in the soil, and Ashby and Beadle [3] reported that in the culture experiments with the addition of NaCl and KCl, *Atriplex* plants tended to increase chloride contents and osmotic pressures of the expressed leaf saps. In the osmotic values of *Salicornia* and *Suaeda* some differences were recognized between the values obtained by the writer and those reported by these foreign investigators, but the values of  $O_{NaCl}/Osm$  percentage found in the present investigation were similar to those reported by them, e. g. above 90% in *Salicornia* (in North America, Steiner [27]) and 81–84% in *Suaeda maritima* (in India, Sen-Gupta [26]).

Comparing the strand dune plants with the halophytes in the osmotic value and the  $O_{NaCl}/Osm$  percentage, we can find a remarkable difference between these two plant groups, that is to say, the former are markedly lower than the latter in these values. Therefore, these two measures seem to be the best of the criteria to distinguish the halophytes from the strand dune plants. On the basis of these measures, moreover, *Salicornia herbacea* and other halophytes can be placed in order concerning halophytic characters.

In the foregoing chapter we took the NaCl-content of the soils where the plant grew as a criterion to classify the strand dune plants and the halophytes. Now we are in a position to add another criterion which is based on the plant character itself, i. e. osmotic value and  $O_{NaCl}/Osm$  percentage of the plant sap.

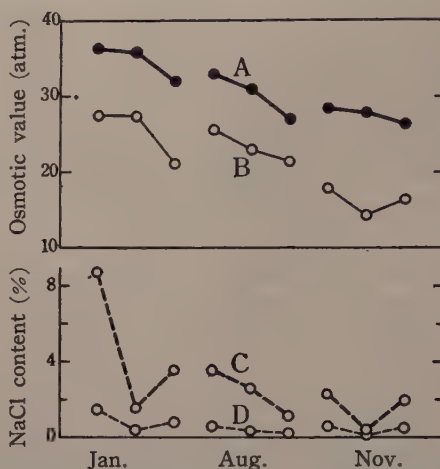


Fig. 5. *Statice japonica*. Osmotic value and NaCl content of expressed saps, and NaCl content of the soil. A, B, C and D indicate the same in Fig. 4.

### III. Effects of salt on germination and growth

Brenner's artificial sea water was diluted with tap water into the following concentrations and used for the germination and growth experiments:

Diluted solutions	Total salt concentration in %	NaCl concentration in %
1/1-solution	3.13	2.02
3/4- "	2.34	1.52
1/2- "	1.57	1.01
1/4- "	0.78	0.51
1/8- "	0.39	0.25
1/16- "	0.20	0.13
1/32- "	0.10	0.06

#### 1. Strand dune plants

Seeds of *Calystegia Soldanella* and *Lathyrus japonicus* collected on the sandy seashore of Kugenuma were used, and they were appropriate for this purpose as they were easy to be collected in sufficient amount for the test and germinated rapidly all together. Seeds of *Zea Mays* L. and *Pharbitis Nil* Chois. were also used for comparison.

Effects of salt on the germination, on the growth immediately after germination and on the growth of the seedlings in cotyledon stage were investigated.

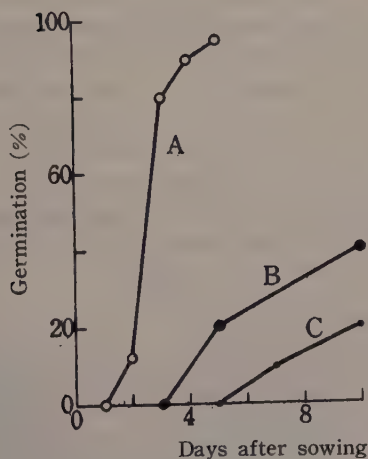


Fig. 6. Effect of salt solutions on the germination of *Calystegia Soldanella*. A: tap water, 1/32-1/16- and 1/4-artificial sea water. B: 3/4-artificial sea water. C: 1/1-artificial sea water.

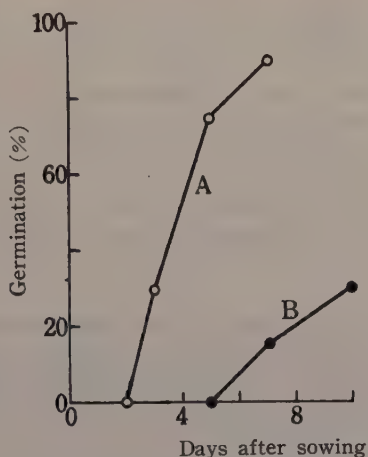


Fig. 7. Effect of salt solutions on the germination of *Lathyrus japonicus*. A: tap water, 1/32-, 1/16- and 1/4-artificial sea water. B: 3/4-artificial sea water.

### A. Effect of salt on the germination

The germination was tested on either filter paper or absorbent cotton; these were respectively moistened by tap water, 1/32-, 1/16-, 1/4-, 3/4- and 1/1-solutions, being kept in Petri dishes.

*Calystegia Soldanella* gave about 95% germination in tap water, 1/32-, 1/16- and 1/4-solutions 5 days after sowing, and showed retarded germination in higher concentrations: in 3/4-solution about 20% germination after 5 days and about 40% after 10 days, and in 1/1-solution about 10% after 7 days and about 20% after 10 days (Fig. 6).

*Lathyrus japonicus* had generally a little smaller germination rate than the *Calystegia*. In tap water, 1/32-, 1/16- and 1/4-solutions, the germination rates were about 30% 3 days after sowing, about 75% after 5 days and about 90% after 7 days. In high concentrated solutions marked retardation was observed in germinations. In 3/4-solution only 15% of the seeds were germinated after 7 days, and about 30% after 10 days. In 1/1-solution no germination occurred even after 10 days (Fig. 7).

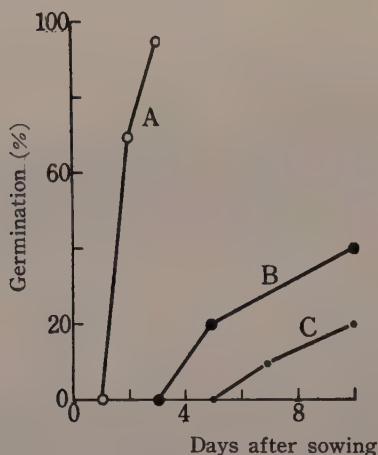


Fig. 8. Effect of salt solutions on the germination of *Zea Mays*.

A: tap water, 1/32-, 1/16- and 1/4-artificial sea water.

B: 3/4-artificial sea water.

C: 1/1-artificial sea water.

*Zea Mays* made about 95% germinations in tap water, 1/32-, 1/16- and 1/4-solutions 3 days after sowing, and in 3/4- and 1/1-solutions retarded germinations similar to the case of *Calystegia Soldanella* were observed (Fig. 8).

As stated above, the seeds of *Calystegia Soldanella* and *Lathyrus japonicus* germinated in the presence of a small quantity of salt (1/32-, 1/16- and 1/4-artificial sea water) just as well as without any salt (tap water), and hardly did in the culture solutions with high concentrations (3/4- and 1/1-artificial sea water). This was more or less the same as in the case of *Zea Mays*.

### B. Effect of salt on the growth immediately after germination

Seeds were sown in the sands which were moistened by tap water, 1/32-, 1/16-, 1/8-, 1/4-, 1/2-, 3/4- and 1/1-solutions and kept in deep dishes, and the growing roots were measured in length certain days after sowing.

#### *Calystegia Soldanella* and *Lathyrus japonicus*

In *Calystegia Soldanella* 5 and 10 days after sowing and in *Lathyrus japonicus* 10 and 15 days after, the lengths of the roots of the seedlings were measured. As an example, mean values measured in a test are shown in Table 25. The tests were conducted several times and from the results, we could find out that

TABLE 25

Root lengths of *Calystegia Soldanella* and *Lathyrus japonicus* measured 5, 10 and 15 days after sowing in various concentrations of artificial sea water (Unit, cm.)

Solutions	<i>Calystegia Soldanella</i>		<i>Lathyrus japonicus</i>	
	after 5 days	after 10 days	after 10 days	after 15 days
Tap water	1.5cm	3.8cm	1.4cm	2.8cm
1/32-solution	1.5cm	3.8cm	1.4cm	2.8cm
1/16-solution	1.4cm	3.6cm	1.2cm	2.6cm
1/8-solution	1.3cm	3.4cm	1.1cm	2.4cm
1/4-solution	1.0cm	2.7cm	0.9cm	1.8cm
1/2-solution	0.5cm	1.0cm	0.6cm	1.0cm
3/4-solution	+	+	+	+
1/1-solution	0	+	0	0

+ indicates a little growth

TABLE 26

Root lengths of *Zea Mays* and *Pharbitis Nil* measured 5 and 10 days after sowing in various concentrations of artificial sea water (Unit, cm.)

Solutions	<i>Zea Mays</i>		<i>Pharbitis Nil</i>	
	after 5 days	after 10 days	after 5 days	after 10 days
Tap water	3.5cm	10.8cm	2.6cm	5.5cm
1/32-solution	3.5cm	10.8cm	2.6cm	5.5cm
1/16-solution	3.3cm	10.5cm	2.4cm	5.3cm
1/8-solution	3.2cm	10.0cm	2.3cm	4.6cm
1/4-solution	2.3cm	7.5cm	1.4cm	3.1cm
1/2-solution	1.0cm	3.0cm	0.9cm	1.4cm
3/4-solution	+	+	+	+
1/1-solution	0	+	0	0

+ indicates a little growth

both *Calystegia Soldanella* and *Lathyrus japonicus* grew in 1/32-solution just as well as in tap water, but their growth was retarded somewhat in 1/16- and 1/8-solutions and clearly in 1/4- and 1/2-solutions, while in 3/4- and 1/1-solutions their growth scarcely occurred.

#### *Zea Mays* and *Pharbitis Nil*

Similar tests were repeated several times with the seeds of *Zea Mays* and *Pharbitis Nil*, and the lengths of the roots were determined 5 and 10 days after sowing. As seen in Table 26, the results were very similar to those found in the strand dune plants discussed above.



Further experiments were conducted. Seeds were previously sown in the sands which were moistened by tap water, and when the roots had grown to a certain length, they were transplanted into deep containers filled with sands moistened by tap water and artificial sea water in various concentrations. The growth of the roots in length was determined certain days after transplanting.

*Calystegia Soldanella* and *Lathyrus japonicus*

Five and ten days after transplanting the seedlings of *Calystegia Soldanella* with roots of 1 cm. in length and of *Lathyrus japonicus* with roots of 0.5 cm. in length, the lengths of their roots were re-examined. As an example, mean values measured in a test are shown in Table 27, where the length of the roots indicates the total length on those dates. To get the root growth itself after transplanting,

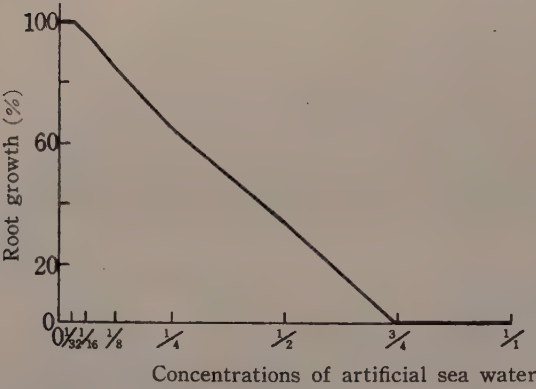


Fig. 9. Effect of salt solutions on the root growth of *Calystegia Soldanella*.

1 cm. in *Calystegia* or 0.5 cm. in *Lathyrus* must be subtracted from the root lengths shown in the table. The tests were repeated several times and from the results it was observed that the roots of both species grew after transplanting in 1/32-solution just as well as in tap water, and the growth diminished gradually with increase of concentration of the solutions. In 3/4- and 1/1-solutions almost no growth was measured in root length after the transplanting. Fig. 9 illustrates

TABLE 27

Root lengths of *Calystegia Soldanella* and *Lathyrus japonicus* measured 5 and 10 days after setting into various concentrations of artificial sea water (Unit, cm.)

Solutions	<i>Calystegia Soldanella</i>		<i>Lathyrus japonicus</i>	
	after 5 days	after 10 days	after 5 days	after 10 days
Tap water	3.9cm	5.8cm	2.9cm	4.5cm
1/32-solution	3.9cm	5.8cm	2.9cm	4.5cm
1/16-solution	3.7cm	5.6cm	2.6cm	4.3cm
1/8-solution	3.5cm	4.7cm		
1/4-solution	2.9cm	4.4cm	2.0cm	3.0cm
1/2-solution	2.0cm	3.0cm		
3/4-solution	1.0cm	1.0cm	1.0cm	1.0cm
1/1-solution	1.0cm	1.0cm	1.0cm	1.0cm

TABLE 28

Root lengths of *Zea Mays* and *Pharbitis Nil* measured 5 days after setting into various concentrations of artificial sea water (Unit, cm.)

Solutions	<i>Zea Mays</i>	<i>Pharbitis Nil</i>
Tap water	5.2cm	4.1cm
1/32-solution	5.2cm	4.1cm
1/16-solution	5.0cm	3.7cm
1/8-solution		3.4cm
1/4-solution	3.5cm	3.0cm
1/2-solution		2.1cm
3/4-solution	1.0cm	1.0cm
1/1-solution	1.0cm	1.0cm

the growth of *Calystegia* roots, showing the increase of root length in percentage to the maximal growth in tap water.

In regard to the results of these experiments, attention must be paid to the fact that tap water (without any salt) and 1/32-solution were most favourable for the growth of roots of *Calystegia Soldanella* and *Lathyrus japonicus* and that better growth was never seen when more salt was added in the culture solutions.

#### *Zea Mays* and *Pharbitis Nil*

Similar tests were carried out with the seeds of *Zea Mays* and *Pharbitis Nil* which were transplanted when the roots had grown up to 1 cm. length, and the lengths of the roots were measured 5 days after the transplanting. As seen in Table 28, the results were very similar to those found in *Calystegia Soldanella* and *Lathyrus japonicus*

#### C. Effect of salt on the growth of the seedlings of *Calystegia Soldanella* in the cotyledon stage

Seeds of *Calystegia* were sown in the sands which were moistened by tap water and after germination the seedlings were continuously cultured with tap water for 2 to 3 weeks. Then, these seedlings in the cotyledon stage were transferred into water-culture using tap water or variously diluted artificial sea water. In tap water, 1/32-, 1/16- and 1/8-solutions, their growth normally continued in appearance and the leaves developed, but the root length was not measured. In 1/4-solution, their growth was poor and sometimes cotyledons turned yellow. In 1/2-, 3/4- and 1/1-solutions injury occurred badly, and such high concentrations killed the seedlings sooner or later. The osmotic values determined with plant saps expressed from the whole plant bodies were 7.4 atm. in the seedlings cultured for over 1 month in a sand-culture with tap water, and 8.3 atm. in the seedlings cultured for over 2 months in a sand-culture using Sachs' solution. It might be supposed that the osmotic value of the young seedlings which were used in the above-mentioned experiment had to be, though it was not determined actually, a little lower than the values measured (7.4 or 8.3 atm.), because the younger plant has generally low osmotic value than the older

one. The osmotic value of 1/4-artificial sea water itself being 4.8 atm., it might reasonably be supposed that the seedlings which had been cultured for 2 to 3 weeks in tap water could tolerate 1/8-artificial sea water with normal growth, and moreover as the osmotic values of the plant saps increased generally with age of seedlings, the critical concentration of artificial sea water against which seedlings could tolerate became a little higher with the growth of seedlings.

## 2. Halophytes

The seeds of *Suaeda japonica* which were collected on the seashore of Obase (1 in Fig. 1) and seeds of *Statice japonica* which were collected in Hiroshima (7 in the same figure) were used for experiments to clarify the effect of salt on the germination and growth of the halophytic plants.

### A. Effect of salt on the germination of seeds

The germination of the *Suaeda* seeds was tested on sheets of filter paper which were moistened respectively by tap water, 1/32-, 1/16-, 1/8-, 1/4-, 1/2-, 3/4- and 1/1-solutions and kept in Petri dishes.

With 1/8- and 1/4-solutions they gave about 55 % germinations 5 days after sowing and about 80 % after 10 days. With tap water, 1/32- and 1/16-solutions, rather slightly retarded germinations were observed in earlier stages. Ten days after sowing, however, they gave under these conditions about 80 % germinations. With 1/2-, 3/4- and 1/1-solutions also fairly retarded germinations were observed in earlier stages and it reached about 70-75 % after 15 days (Fig. 10). In *Suaeda japonica*, unlike the case of the above-mentioned strand dune plants, in earlier stages retarded seed germination occurred in lower concentrations of salt than 1/8-solution. In later stages *Suaeda* indicated in 3/4- and 1/1-solutions higher germination percentages than the strand dune plants did.

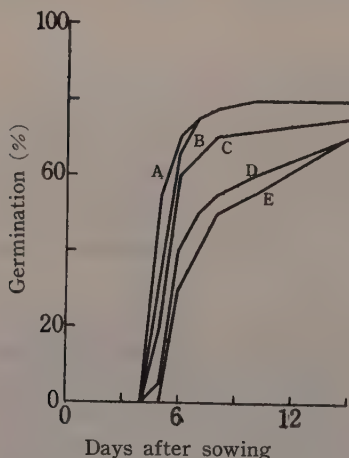


Fig. 10. Effect of salt solutions on the germination of *Suaeda japonica*. A: 1/4- and 1/8-artificial sea water. B: tap water, 1/32- and 1/16-artificial sea water. C: 1/2-artificial sea water. D: 3/4-artificial sea water. E: 1/1-artificial sea water.

### B. Effect of salt on the growth of seedlings

*Statice japonica* and *Suaeda japonica* were used in this experiment. Seeds of these plants were previously sown on filter paper which was moistened by tap water and kept in Petri dishes. Immediately after germination they were transferred into water-culture using tap water, 1/32-, 1/16-,

1/8-, 1/4-, 1/2-, 3/4- and 1/1-solutions. After the solution-cultures for about 2 to 3 weeks the total fresh weights of the seedlings were measured with a torsion

balance because of a difficulty of exact measurement of the root length. Results of a test, in which the seedlings were kept in the solution-culture for 3 weeks, are shown in Table 29 and in Fig. 11, as an example. In the case of *Statice*, a 1/8-solution was the most favourable for the growth of the seedlings, and in the case of *Suaeda* a 1/2-solution was the most favourable, whereas in lower as well as in higher concentrations than these solutions the growth of both species was depressed.

TABLE 29

Fresh weights of 10 plants of *Suaeda japonica* and *Statice japonica* measured 3 weeks after setting into various concentrations of artificial sea water (Unit, mg.)

Solutions	<i>Suaeda japonica</i> immediately after germination	<i>Statice japonica</i>	
		immediately after germination	cotyledon stage
Tap water	34 mg	60 mg	75 mg
1/32-solution	37 mg	71 mg	82 mg
1/16-solution	40 mg	72 mg	83 mg
1/8-solution	43 mg	74 mg	88 mg
1/4-solution	45 mg	73 mg	91 mg
1/2-solution	46 mg	69 mg	89 mg
3/4-solution	44 mg	61 mg	77 mg
1/1-solution	40 mg	51 mg	66 mg

In *Statice japonica*, sometimes the seedlings were kept longer (for about 1 to 2 weeks) in the preculture on filter paper with tap water, and on reaching the cotyledon stage uniform seedlings were selected and transferred into water-cultures. In such a case the most favourable concentration for seedling growth shifted somewhat to higher concentrations. From a result of the experiments, in which the seedlings of *Statice japonica* were kept in the preculture with tap water for 1 week after germination and in the solution-cultures for 3 weeks (Table 29 and Fig. 11), we can find that in the cotyledon stage a 1/4-solution was the most favourable for their growth.

These tests were repeated several times with varying periods of preculture in tap water and of the test-solution culture, and thereby some

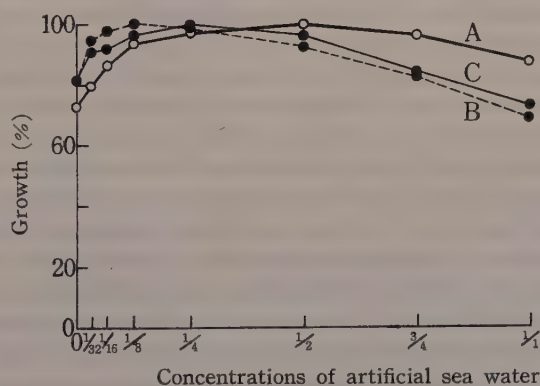


Fig. 11. Effect of salt solutions on the growth of *Suaeda japonica* and *Statice japonica*. A: *Suaeda japonica*. B: *Statice japonica* (immediately after germination). C: *Statice japonica* (cotyledon stage).



fluctuations were found in the rate of growth. In any case, the maximum growth was always observed in the presence of some amount of salt but not in the absence of it.

Further experiments were carried out in *Statice japonica*. The seedlings were, after a long preceding sand-cultures with tap water, set for a long period in sand-cultures using Knop's culture solution alone or with adding NaCl in concentrations of 0.05, 0.1, 0.2, 0.4 and 0.8%. In these tests also better growth of the seedlings occurred in the presence of some amount of NaCl than in the absence of it.

Montfort and Brandrup [18] reported that the maximum growth of *Salicornia herbacea* occurred with 3/4-artificial sea water and that of *Aster tripolium* with 1/8-artificial sea water. In the present investigation, though the growth was not measured in the same way, the writer has found that artificial sea water in 1/8-1/4 concentrations was the most favourable for the growth of *Statice japonica* and that in 1/2, for *Suaeda japonica*. It is very interesting that the growth maximum of *Statice japonica* and of *Suaeda japonica* occurred in case of presence of some salt amount which was smaller than the amount for the best growth of *Salicornia herbacea*. From this fact, we may settle the order in halophytic characters of these three species as follows:

*Salicornia herbacea* > *Suaeda japonica* > *Statice japonica*.

Between the results obtained for the strand dune plants and those for the halophytes there were remarkable differences. In the germination test, the seeds of a halophyte *Suaeda japonica*, unlike the case of the strand dune plants, *Calystegia Soldanella* and *Lathyrus japonicus*, germinated more rapidly in the presence of some amount of salt (artificial sea water in concentrations of 1/8 and 1/4) than in the absence or the presence of smaller amount of salt (tap water, and artificial sea water in concentrations of 1/32 and 1/16), though 10 days after sowing they all gave about 80% germinations. In the presence of a larger amount of salt (artificial sea water in concentrations of 3/4 and 1/1), *Suaeda japonica* gave a higher percentage of germination (Fig. 10) than *Calystegia Soldanella* (Fig. 6) and *Lathyrus japonicus* (Fig. 7). In the growth experiment the maximum growth of the seedlings of *Calystegia* and *Lathyrus* occurred in the absence of salt (in tap water) or in the presence of very small amount of salt (1/32-artificial sea water), which is quite similar to the case of non-halophytic plants, such as *Zea Mays* and *Pharbitis Nil*. In *Statice japonica* and *Suaeda japonica*, however, the maximum growth of the seedlings, though the growth was not measured in the same way, occurred in the presence of some amount of salt, i.e. in *Statice* with artificial sea water in concentrations of 1/8-1/4 and in *Suaeda*, 1/2, and in the absence of salt or in the presence of a small amount of salt, the growth of the seedlings was somewhat depressed. In the presence of larger amount of salt, 3/4- and 1/- artificial sea water, seedlings of strand dune plants *Calystegia Soldanella* and *Lathyrus japonicus* scarcely grew, while even in 1/1- artificial sea water seedlings of *Suaeda japonica* could grow pretty well and those of *Statice japonica*, in some degree.

Although the experiments have not been carried out with many species, we may regard as an important distinction between the strand dune plants and the

halophytes the characters whether better growth occurs or not in the presence of some amount of salt than in the absence of it. Thus as the third important criterion to distinguish the halophytes from the strand dune plants in halophilic characteristics, we now have this marked difference in the effect of salt on the growth between these two kinds of the plants.

## DISCUSSION

Concerning ecological studies on the strand dune plants and the halophytes from three points of view; the NaCl content of soils, the osmotic value and NaCl content of the plant saps, and the effect of salt on the germination and growth of plants, the writer has discovered marked differences in the results of these two plant groups. So their halophilic characters should be discussed from these three points of view.

NaCl contents in the soils which were covered with the strand dune vegetation are markedly lower than those in the soils which were covered with the halophytic vegetation. As seen in Tables 1~13, most of the soils collected from the strand dune vegetation, with few exceptions, contain NaCl in 0.003~0.004 % and the soils collected at the place far from the tide line, only in traces. On the contrary, the soils collected in the halophytic vegetations contain, as seen in Tables 14~18, far more NaCl, namely about 100~500 times of the NaCl content of the soils in the strand dune vegetation in the quantity. Such marked difference in NaCl content between the soils must be adopted as one of the important standards to distinguish between the strand dune plants and the halophytes.

Kearney [14] reported that some of the species which grew normally on the seashore strands were found on the dunes of Lake Michigan. Also in Japan it is well known that some species growing normally on the sandy seashores can be found on some inland sandy soils far from the sea, e.g. *Carex pumila* growing among other mesophytic herbs at Shôbugahama, the sandy shore of Lake Chûzenji (21 in Fig. 1) near Nikko, *Calystegia Soldanella* growing on the sandy shore of Lake Biwa (10) and some of the strand dune plants growing on the inner dunes far from the sea at Ôta, Ibaraki Prefecture (20).

In the Botanical Garden of the University of Tokyo, *Carex Kobomugi*, *Carex pumila*, *Calystegia Soldanella*, *Tournefortia sibirica* and *Lathyrus japonicus* were transplanted and grew well on the sandy soils which contained NaCl only in a trace (pH 6.2). Loss on ignition was determined with both of the sandy soils covered with vegetation which were collected on the seashore of Taito and in the Botanical Garden, and the results were 0.4~0.7 % in the former and about 4 % in the latter. Roughly speaking, loss on ignition may be considered as organic matter contained in the soil, and so we may say that not only in NaCl content and soil reactions but also in organic matter content, the sandy soil of the Botanical Garden differs from those of the seashore of Taito.

From the ecological point of view, it is very interesting that some of the natural inhabitants on the seashore strand can also be found on some inland sandy soils far from the sea. It may easily be considered that the strand dune plants

growing on the seashore soils with low NaCl content must be able to grow on the inland sandy soils. Still it is not reasonable to suppose that all the strand dune plants can grow on the inland sandy soils, because the halophilic characters should never be considered to be in the same grade throughout the strand dune plants (Takada (32)).

Inquiring after the osmotic values and the  $O_{NaCl}/Osm$  percentage, we find remarkable differences between the strand dune plants and the halophytes as shown in Tables 19~24 and Fig. 12. From the fact that the  $O_{NaCl}/Osm$  percentages of the halophytes are far larger than those of the strand dune plants, it

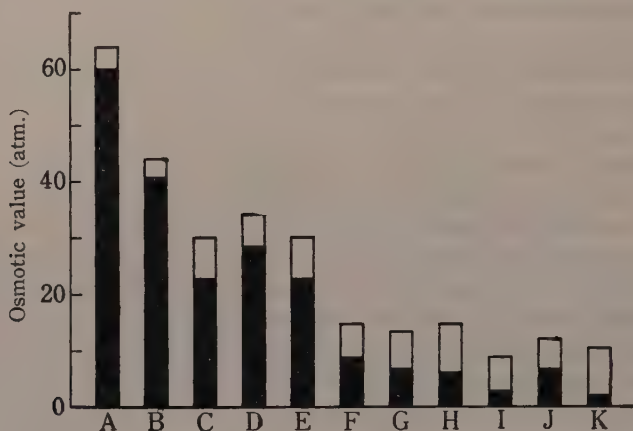


Fig. 12. The osmotic value of the halophytes and the strand dune plants. Total height of the column shows the total osmotic value (in atm.) and the black part shows NaCl contained in plant saps (in atm.). A: *Salicornia herbacea* (Nov.). B: *Salicornia herbacea* (Aug.). C: *Statice japonica* (Aug.). D: *Suaeda maritima* (Aug.). E: *Suaeda japonica* (Aug.). F: *Carex Kobomugi* (Aug.). G: *Tournefortia sibirica* (Aug.). H: *Carex pumila* (Aug.). I: *Ischaemum antheophoroides* var. *eristachyum* (July). J: *Glehnia littoralis* (Aug.). K: *Lathyrus japonicus* (Aug.).

is suspected that NaCl in plant saps plays more important parts in the increase of osmotic value in the halophytes than in the strand dune plants. Attention must be paid, however, not to suppose that the large  $O_{NaCl}/Osm$  percentage always signifies directly the large absolute amount of NaCl in plant saps; when the osmotic value is not so high, even the small absolute amount of NaCl in plant saps may represent a large  $O_{NaCl}/Osm$  percentage. Even nonhalophytes contain some, but not large, amount of NaCl and NaCl in their plant saps can play a certain part in the shift of the osmotic value. It may be said that in case of high osmotic value and large  $O_{NaCl}/Osm$  percentage—naturally of a large absolute amount of NaCl in plant sap,—halophilic characters of the plant are strong. Therefore, to discuss the halophilic characters of the plant, we must care for both the osmotic value and the  $O_{NaCl}/Osm$  percentage. From the results of the experiments, it can be said that the strand dune plants are clearly weaker in the halophilic character than the halophytes, because the osmotic



values are markedly lower and the  $O_{NaCl}/Osm$  percentages are also remarkably smaller in the former than in the latter. Comparing the halophytes with each other in these values, we find out that e.g. *Salicornia herbacea* indicated higher osmotic value and larger  $O_{NaCl}/Osm$  percentage than three other halophytic species, *Statice japonica*, *Suaeda maritima* and *Suaeda japonica*. So it is conclusive that *Salicornia herbacea* is the strongest in the halophytic characters of the halophytes. Similarly we can find in the strand dune plants that the values of *Carex Kobomugi*, *Carex pumila*, *Glehnia littoralis* and *Tournefortia sibirica* are somewhat higher than those of *Ischaemum antheophoroides* var. *eristachyum* and *Lathyrus japonicus*, but it may be difficult to make clear the difference in the halophilic characters of these plants from these values alone.

The writer found on the investigated sandy seashores that *Carex Kobomugi* sometimes grew in soils which were extremely rich in NaCl (0.099 and 0.056 %) and that the habitat of *Wedelia prostrata* was ordinarily limited to the area near the tide line. On the other hand, some species of the strand dune plants were found on some sandy soils far inland. From these facts we may suppose that some differences in the halophilic characters among the strand dune plants must exist, though the writer could hardly find out any clear distinction in halophilic characters, such as in osmotic values and  $O_{NaCl}/Osm$  percentage, among them. Recently, based on the Na:K ratio in the expressed plant sap, Takada [32] classified the strand plants into three groups: euhalophytes (*Atriplex subcordata* var. *japonica*, *Suaeda asparagoides* and *Aster tripolium*), oligohalophytes (*Wedelia prostrata*, *Phellopterus littoralis* (= *Glehnia littoralis*) and *Phragmites communis*) and nonhalophytes (*Vitex rotundifolia*, *Calystegia Soldanella* and *Lathyrus maritimus* (= *Lathyrus japonicus*). Halophilic differentiation of the strand dune plants, therefore, appears to be demonstrated easier by such a method as adopted by Takada. However, the osmotic values and the  $O_{NaCl}/Osm$  percentages seem to be more fundamental to distinguish between the strand dune plants and the halophytes, although these measures appear to be somewhat too broad to classify the subdivision of the strand dune plants.

Effect of salt on the germination and growth was investigated in a few species. The maximum growth immediately after the germination of strand dune plants, *Calystegia Soldanella* and *Lathyrus japonicus*, occurred in the absence of salt (tap water), or in the presence of a very small amount of salt (1/32-artificial sea water) and the growth diminished gradually with increase of the salt amount added. This was quite similar to the case of *Zea Mays* and *Pharbitis Nil*. On the contrary, the maximum growth of halophyte seedlings occurred in the presence of some amount of salt; the optimum concentration was in *Statice japonica* artificial sea water of 1/8-1/4, and in *Suaeda japonica*, 1/2, and in the absence of salt fairly depressed growth of them was observed. The *Calystegia Soldanella* and *Lathyrus japonicus* belong to nonhalophytes (cf. Takada [32]), and somewhat different results might be expected in the seeds of oligohalophytes. Hereto, the writer tried the germination experiment using the seeds of *Carex Kobomugi* and *Carex pumila* etc. Unfortunately, none of them germinated. The growth experiments were not conducted with the seeds of the strand dune plants which belonged to oligohalophytes, but as to the soil salinity of



habitat, the osmotic value and  $O_{NaCl}/Osm$  percentage, *Glehnia littoralis* which was included in oligohalophytes after Takada's classification [32] is quite similar to the other strand dune plants, but there was marked difference between this plant and the halophytes. Although so far we have only the experimental data restricted in a few species, whether or not in the presence of some amount of salt better growth occurs than in the absence of any salt, may be regarded as one of the most essential criteria to distinguish the halophytes from the strand dune plants. From this point of view, it is needless to say that the halophytes are much stronger in the halophilic characters than the strand dune plants.

From the discussions on the halophilic characters of strand dune plants and halophytes based on the three criteria, the soil salinity of the habitat, the osmotic value and  $O_{NaCl}/Osm$  percentage, and the effect of salt on the growth, and the consideration of the fact that some of the strand dune plants grow on some sandy soils far inland, it is reasonably concluded that a decided difference exists in the halophilic characters between the halophytes and the strand dune plants. The latter are much weaker in the halophilic characters than the halophytes; the salinity of the soils and the osmotic value are markedly lower and the  $O_{NaCl}/Osm$  percentage is also markedly smaller in the strand dune plants than in the halophytes, and the maximum growth of the strand dune plants, though only in experiments with two species, occurs in the absence, or in the presence of a very small amount, of salt, while the maximum growth of the halophytes occurs in the presence of some amount of salt and the growth is reduced if without salt.

Discussions on the halophytic characters of the halophytes from the same points of view may induce a conclusion that *Salicornia herbacea* is superior in the halophytic characters to *Statice japonica*, *Suaeda maritima* and *Suaeda japonica*, because the salinity of soils, the osmotic value and  $O_{NaCl}/Osm$  percentage are the highest in *Salicornia herbacea* of these four species and moreover the concentration of the artificial sea water permitting the maximum growth is considerably higher in *Salicornia herbacea* (Montfort and Brandrup [18, 19]) than in *Suaeda japonica* and *Statice japonica*, though the growth was not measured in the same way. Between *Suaeda japonica* and *Statice japonica* there was not much difference in the halophytic characteristics discussed above. However, the concentration permitting the maximum growth is higher in *Suaeda japonica* than in *Statice japonica* and this may suggest that some differences may exist in the grade of the halophytic character between these two species. From these facts it may naturally be said that among the halophytes differences exist in the grade of the halophytic characters. Among the strand dune plants, however, the writer has found no distinct differences in the grade of halophilic characters, and those would be studied more conveniently by the method as adopted by Takada [32].

From the results obtained from these investigations, the writer suggests that in discussing the halophilic characters of the plants from the ecological point of view, we must pay attention to the three points: soil salinity of the habitat, the osmotic value and  $O_{NaCl}/Osm$  percentage, and the amount of salt in the culture solutions permitting the maximum growth, and that the halophilic characters are to be discussed synthetically from these three points of view.

## SUMMARY

From the ecological studies on the strand dune plants and halophytes in Japan, the following results were achieved.

1. Soils which were covered with the strand dune vegetation contained generally a small amount of NaCl, 0.003~0.004 % on a dry weight basis, or the soils far from the tide line, only a trace of the salt. On the contrary, the soils which were covered with the halophytic vegetation contained about 100~500 times the quantities of NaCl which were found in the soils mentioned above.

2. The osmotic values of the plant saps of strand dune plants were markedly lower than those of the halophytes, and the values of the ratio of the estimated osmotic value due to NaCl in plant saps to the total osmotic value determined by the cryoscopy method ( $O_{NaCl}/Osm$  percentage) were also remarkably smaller in the strand dune plants than in the halophytes.

3. Immediately after the germination, the maximum growth of the seedlings of the strand dune plants, *Calystegia Soldanella* and *Lathyrus japonicus*, occurred in the absence of salt (in tap water) or in the presence of a very small amount of salt (1/32-artificial sea water), and the growth diminished in the presence of a larger amount of salt. This was quite similar to the case of *Zea Mays* and *Pharbitis Nil*. The maximum growth of the halophytes, however, was observed in the presence of some amount of salt (in *Statice japonica*, artificial sea water in concentrations of 1/8-1/4, and in *Suaeda japonica*, 1/2), and in the absence of salt the growth of these halophytes was fairly depressed.

4. From the above-mentioned results, we may conclude that there is a marked difference in the halophilic characters between the strand dune plants and the halophytes: namely, the former are much weaker in the halophilic characters than the latter.

5. The results obtained in the halophytes revealed the existence of a considerable difference in the halophytic characters among these plants. The halophytic characters of *Salicornia herbacea* are stronger than those of *Statice japonica*, *Suaeda maritima* and *Suaeda japonica*, and among these three species *Suaeda japonica* is a little superior in the halophytic characters to *Statice japonica*.

6. It may be suggested that, in discussing the halophilic characters of the plant from the ecological point of view, attentions must be paid to the three following characteristics: the salinity of habitat, the osmotic value and NaCl content of plant saps, and the amount of salt in the culture solution which permits a maximum growth of the plant.

The writer wishes to express his sincere thanks to Dr. H. Nakano, the former Professor of the University of Tokyo, for his kind guidance and valuable advice given throughout this investigation. He wishes also to express his thanks to Dr. M. Honda, Emer. Professor of the University of Tokyo, for his valuable guidance in systematics and to Dr. M. Monsi, Professor of the University of Tokyo, for his helpful advice. He is also indebted to Mr. N. Satomi, Lecturer of Kanazawa University, for his assistance.

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### Explanation of Plates

Photo 1. View of the seashore in the vicinity of Kanazawa (Fig. 1-11). The dominant species is *Carex Kobomugi*.

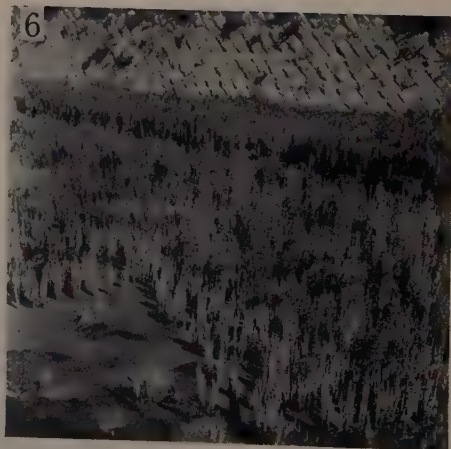
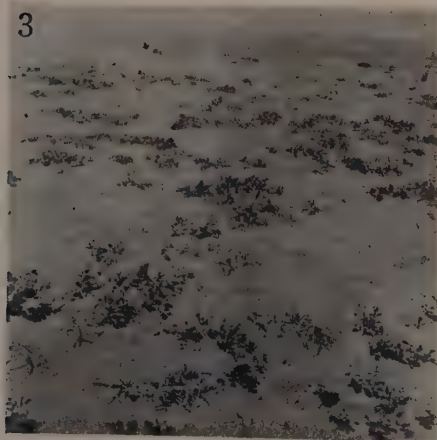
Photo 2. View of the seashore of Usa, Kôchi Prefecture (Fig. 1-2), where *Vitex rotundifolia* dominates.

Photo 3. View of the sandy strand at the river-mouth of the Niyodo River, Kôchi Prefecture (Fig. 1-3). Main plant species are *Carex Kobomugi* and *Glehnia littoralis*.

Photo 4. View of the sandy strand at the river-mouth of the Niyodo River, Kôchi Prefecture (Fig. 1-3). *Carex Kobomugi* is the main sand dune plant.

Photo 5. Plant community of *Statice japonica* at Takihama, Ehime Prefecture (Fig. 1-5).

Photo 6. *Salicornia herbacea* community in a salt marsh at Takuma, Kagawa Prefecture (Fig. 1-6).



# DEVELOPMENT OF VEGETATION IN RELATION TO SOIL FORMATION IN THE VOLCANIC ISLAND OF OSHIMA, IZU, JAPAN

YASUHIKO TEZUKA<sup>1)</sup>

## I. INTRODUCTION

Since the succession theory was systematized by Clements [9] dynamics of vegetation has been a principal subject for plant synecology [7, 15, 48, 63, 66]. There have been many investigations on the plant succession on rocks, sand dunes, bogs, recessional moraines, mud-flows and other bare lands. But many, if not most, of such works have mainly been concerned with description of successional sequences and species composition of plant communities developing from pioneer stage to climax stage. To understand the close relationship between the vegetation and its environment fundamentally, the study of succession provides further approaches in the following. (1) Quantitative study of the correlations between vegetational and environmental changes during the progress of succession, since the replacement of one community by another depends upon the environmental changes brought about by the reaction of the occupying vegetation [9]. (2) Elucidation of specific responses of each species to the environment based on its autecology. (3) Analyses of the competitive or cooperative relations among main species of successive communities. Approach (3) can, however, be accomplished with successive performances of approaches (1) and (2).

Rather few authors have been concerned with these approaches because of the complexity involved in the environment-vegetation relations. Studies in approach (1) have recently been pursued by such workers as Crocker and Dickson [12], Crocker and Major [13], Dickson and Crocker [16, 17, 18] and Olson [47], with special reference to edapho-vegetational relations. Difficulties accompanied with this phase of ecology have been discussed in detail by Billings [2, 3], Crocker [11], Major [36], Beadle [1], Sjörs [60], Jenny [25] and so on. On the other hand, approach (2) has already been made for various aspects of Piedmont vegetation [5, 6, 32, 33, 49]. Monsi and Oshima [43] have tried to analyze theoretically the successional processes from grassland to forest and from sun-tree forest to shade-tree forest with regard to light factor, on the basis of the dry matter production. A similar trial has also been made by Nomoto [46] in the analysis of succession process in the beech-oak forest of central Japan. Hogetsu et al. [23] made clear the mechanism of progressive replacement of species in the succession of bog community of Ozegahara considering the changes of edaphic factors through the reaction of plants.

The volcanic island of Oshima, Izu, Japan, provides a superior field available for the study of primary succession, since its volcanic deposits with different ages support various types of seral vegetation.

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The present paper deals chiefly with edapho-vegetational relations, with particular reference to mineral nutrient economy of edapho-vegetational system. A possible explanation for the replacement of one community by another is also presented from the results observed during field surveys.

## II. DESCRIPTION OF THE ISLAND STUDIED

### A. Physiography

The volcanic island of Oshima, which is the largest and northern-most of the Seven Islands of Izu, is situated at Sagami Bay, about 120 km. distant from Tokyo to the south-west, and it is an active double volcano, which belongs to the Fuji Volcanic Zone. This island, having an area of 88 km<sup>2</sup>., is an ellipse in plan, whose major axis running from north-west to south-east is 15 km. in length and the minor one 8.5 km. Outer slope of its elliptical somma, having major axis of 3.2 km., faces loosely the ocean and inner slope which is very steep embraces an atrio where Mt. Mihara (755 m. above sea-level), the central cone of the volcano, towers in the centre (Fig. 1, Pl. 1).

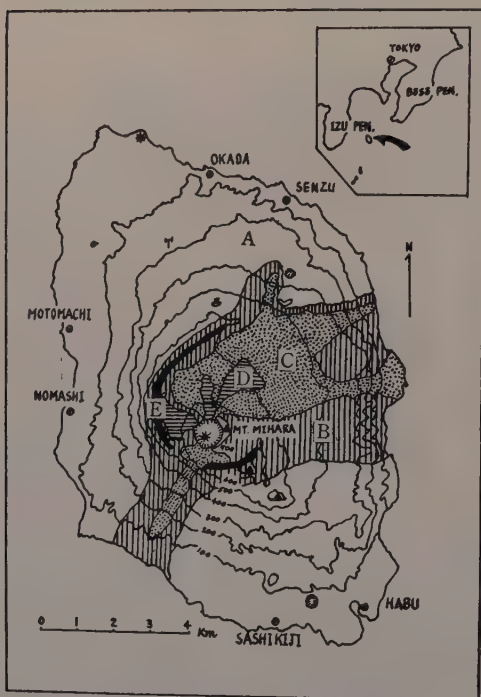


Fig. 1. A map showing the topography and geology of the volcanic island of Oshima.

A: somma ejecta (684?), B: central cone ejecta, C: lava flow (1778), D: lava flow (1950, '51), E: lava flow (B.C. 2000 ?).

sixty million ton of lava flowed into the atrio (Fig. 1 and Pl. 1). The lavas are porous and brittle. Both volcanic ashes and lavas are black in color and of basaltic nature.

Volcanic activity of this island has been considered very old, and historical records show numerous eruptions to the present time. However, ages of the deposits which constitute the present surfaces are comparatively young, of which the oldest is the volcanic ashes presumably ejected about 1200 years ago. In 1778 took place a great eruption, which let flow a large amount of lava across the somma to east and west, and a part of the lava flow reached the ocean. Then a comparatively large eruption occurred in 1950 and 1951, when about

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### B. Climate

Climate of Island Oshima is rather mild under the influence of a branch-current of the Kuroshio. Climatic data which have been recorded at Oshima Meteorological Observatory located at the foot of the northern slope of the somma (193 m. above sea-level), are summarized in Table 1, where temperature in Tokyo is also given for comparison. In this island it is cooler in summer and warmer in winter than in Tokyo, reflecting the characteristic of the oceanic climate. With respect to climatic zone, this island belongs to warm temperate zone. Moreover, much precipitation prevails evenly throughout the year. But it is noteworthy here to denote that in spite of the plentiful precipitation, the wide distribution of barren rocks and soils makes this island considerably dry, and there are neither large valleys nor streams.

TABLE 1.

Climatic data of Oshima, which were obtained from Oshima Meteorological Observatory (193 m. above-sea-level). In this table the temperature of Tokyo was also given for comparison.

Month	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Annual
Mean temp. (°C)	6.0	5.7	8.1	12.6	16.6	19.7	23.8	24.7	22.3	17.3	13.1	8.5	14.9
Mean temp. of Tokyo	3.2	3.9	7.0	12.8	17.2	20.8	25.1	26.4	22.6	16.4	11.0	5.7	14.3
Mean precip. (mm)	110	147	225	213	237	337	225	314	373	437	287	111	3016
Mean relat. humid. (%)	63	63	69	75	81	88	89	87	84	80	75	66	77

### C. Influence of Human Activity

As a principal industry of the inhabitants is forestry, natural forest has been cleared repeatedly over a wide area. Accordingly, the investigation has encountered great difficulties caused by the disturbance of the vegetation by human activity. There, however, remain yet enough large, though scattered, areas of untouched natural vegetations to analyse the characteristics of the seral vegetations.

## III. METHODS OF INVESTIGATION

### A. Vegetation

The present investigation was carried out for three years from 1957. Its first step began with general surveys for classification and mapping of the vegetation types as a basis of further field work. Then, nine stands, each representative for each type, were selected for detailed examination of the vegetation. Of these stands two were situated on "desert" (barren community) and the others on forest. Data were collected by settling quadrats of 10×10 m.. As for

"desert" community, diameters of all colonies of herbs and grasses which appeared in a unit land area were measured. When wet and dry weights of several standard colonies were determined, and plotted against the diameter of colony, a direct proportionality was found between these measures. Basing on this, the cover degree and standing crop of the "desert" community were estimated. Regarding forest stands number of trees and their diameter breast high larger than 2 cm. were measured directly for each species, and then basal area of each species in a unit area was calculated. Height of the vegetation was also measured in each stand. Relative light intensity in the community was determined using two electric photometers [24].

In order to estimate the amount of leaves per unit land area which is useful for the estimates of plant reaction to the soil through leaf fall and for the production of dry matter, ten trees, each belonging to various species and having different diameter breast high, were cut down, then wet weights of their total leaves were weighed immediately. Sub-samples were oven-dried at 80°C for the determination of dry weight.

### *B. Soil*

Soils were sampled at different places of twenty-three including the nine stands chosen for settling the quadrats (see Fig. 2). Soil profiles at the nine stands were observed carefully, soils being sampled for each horizon. Usually, the changes of soils in vertical direction were so gradual that arbitrary collections at 0-10 and 10-20 cm., excluding litter layer, were made at other places than those nine stands. Soils were then air-dried, passed through 2 mm.-sieve and used for physical and chemical analyses.

Apparent density of the soil samples was determined with a 200 ml. glass cylinder. Determination of moisture equivalent was made according to the rapid method of Bouyoucos [20]. Although it has often been pointed out that the values obtained by this method occasionally differ from those obtained by the original method introduced by Briggs and McLane [8], the values thus obtained seem significant for comparative purpose. Mechanical analyses were made by means of sieves with various meshes.

As for chemical properties of soil, ignition loss, total nitrogen (by the micro-Kjeldahl method), total phosphorus (by digestion with sulfuric and nitric acids), etc., were measured. Available nutrients, i.e. ammonia, nitrate, phosphate, potassium and calcium, were extracted with Morgan's solution (10% sodium acetate solution adjusted to pH 4.8 with acetic acid) and analyzed according to the method of Peech and English [51].

Cation exchange capacity was determined by a modification of Schollenberger-Simon's method [59]. Appropriate amount of soil sample was repeatedly leached with 1 N neutral solution of ammonium acetate and then rinsed with 80% methanol until free of ammonia. Exchangeable ammonia was then displaced from the cation exchange complex with 1 N neutral solution of barium chloride and determined colorimetrically with Nessler reagent using a Leitz-electric colorimeter. Cation exchange capacity was expressed as m. equiv. per 100 g. air-dried soil.

### C. Chemical Analyses of Plant Materials

Micro-Kjeldahl method was adopted for measurement of total nitrogen. Dry ashing of about 1 g. of materials was performed in an electric furnace at about 550°C. Of the HCl-extracts of the ashes, total phosphorus, potassium, calcium and magnesium were measured by the ammonium molybdate method (according to Denigè), flamephotometer method (using a Perkin-Elmer-flamephotometer), Ca-oxalate method and EDTA method, respectively.

## IV. GENERAL FEATURES OF THE VEGETATION

The flora of this island is comparatively well known, and more than 600 species of vascular plants have been described [65], a few of which are endemic to this island or to the Seven Islands of Izu.

But a number of the total species have, in all probability, been introduced accidentally or deliberately since the settlement of the inhabitants. In spite of much information on the flora, ecological investigation has scarcely been made. Koizumi [31] made a preliminary survey on the vegetation, whose attention, however, had been rather focused upon the floristical aspects. He recognized the following five vegetational zones: 1. *Pinus Thunbergii* zone, 2. artificial forest zone, 3. mixed deciduous-evergreen forest zone, 4. scrub zone and 5. "desert" zone. His division is useful to some extent at present but unsatisfactory, partly because it was on the weak basis of mere observation of vertical zonation and not on the basis of successional trends, and partly because successional ages of about fifty years have changed the vegetation patterns considerably after his survey.

Therefore, the present author after his intensive surveys divided the vegetations physiognomically into the following five types, of which division was based on the successional trends and soil

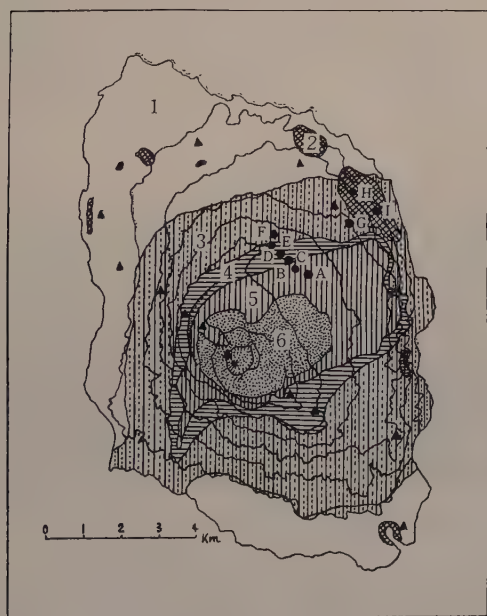


Fig. 2. Vegetation map of Oshima. The borderline as shown in this figure is rather obscure, since the transference of vegetation from one type to another is often continuous. 1: artificial forest, 2: evergreen broad-leaved forest, 3: mixed-deciduous-evergreen broad-leaved forest, 4: scrub, 5: volcanic desert, 6: bare land. Circles with A, B, C, etc. in the figure show the stands where quadrats were settled. Triangles show the sites where soils were sampled.



development (Fig. 2).

1. Artificial forest:— This type of forest is found near the villages at the foot of the somma, where alder (*Alnus Sieboldiana*), Oshima-cherry (*Prunus Lannesiana* var. *speciosa* form. *simpliciflora*) and dogwood (*Cornus controversa*), all of which are deciduous trees, are mainly planted for the fuel. Camellia (*Camellia japonica*) is also planted for the production of camellia-oil. All these species are indigenous. Within the artificial forests just outlined, natural simple forests dominated by *Pinus Thunbergii* can be seen at the seashore having small distributing area.

2. Mixed deciduous-evergreen broad-leaved forest:— Natural forest of this type occupies the vast area of outer slope of the somma, where principal trees are such deciduous ones as *A. Sieboldiana*, *P. Lannesiana* f. *simpliciflora*, *Styrax japonica* var. *iciomotensis*, *C. controversa*, *Stachyurus praecox* var. *Matsuzakii*, etc., some of which constitute dominant layer of the forest, and such evergreen broad-leaved ones as *Camellia japonica*, *Cinnamomum japonicum*, *Neolitsea Sieboldii*, *Eurya japonica* var. *montana*: all these are the constituents of the sub-tree layer. Shrub layer is not well-developed (Fig. 3, Pl. 2). Under the forest canopy a comparatively good cover of grasses, herbs and ferns can be seen.

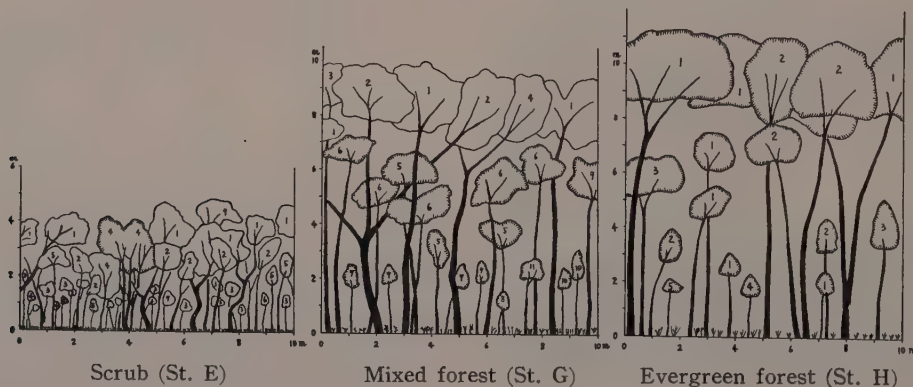


Fig. 3. Dissection of the three representative forests in Oshima. Each diagram represents a plot of 10 m by 5 m.

Outlined deciduous tree,      Fringed, evergreen tree.

Scrub: 1, *Alnus Sieboldiana*; 2, *Weigela glandifolia*; 3, *Ligustrum pacificum*; 4, *Ilex crenata*; 5, *Callicarpa japonica* var. *luxurians*; 6, *Euonymus Sieboldianus*; 7, *Cornus controversa*; 8, *Eurya japonica* var. *montana*; 9, *Prunus Lannesiana* var. *speciosa* form. *simpliciflora*.

Mixed forest: 1, *Styrax japonica* var. *iciomotensis*; 2, *P. Lannesiana* var. *speciosa* form. *simpliciflora*; 3, *C. controversa*; 4, *A. Sieboldiana*; 5, *Neolitsea Sieboldii*; 6, *E. japonica* var. *montana*; 7, *Camellia japonica*; 8, *Cinnamomum japonicum*; 9, *Machilus Thunbergii*; 10, *Gilibertia trifida*; 11, *Otho-dendron japonicum*.

Broad-leaved evergreen forest: 1, *Shiia Sieboldi*; 2, *M. Thunbergii*; 3, *E. japonica* var. *montana*; 4, *N. Sieboldii*; 5, *G. trifida*.

3. Evergreen broad-leaved forest (warm temperate forest):— Although the distributing area of this forest type is very small due to heavy cutting, several small patches can be seen within the mixed forest zone. The greatest of them is located at Senzu, where considerable land area has been protected from cutting as Oshima Natural Park (Pl. 3). The most striking feature of the vegetation is the prominence of *Shiia Sieboldi* and *Machilus Thunbergii* (Fig. 3). The fact that giant trees of these species stand here and there individually along the road near the seashore suggests clearly that this forest type had ever occupied a considerable area of the lower slope.

4. Scrub:— This type of vegetation located near the summit of the somma is characterized by open forest canopy, under which a vigorous growth of herbaceous plants can be seen because of the high light intensity in the community. Dominant species are *A. Sieboldiana* and *Weigela grandifolia*. Height of the forest is less than 5 m. (Fig. 3).

5. "Desert":— The term "desert" as used here is arbitrary since it is not true desert usually found in the regions with minute precipitation. Strictly speaking, "rock desert" or "Petrideserta" after Rübel [53] should be used for the formation in question. This type of vegetation occupies the area of about 10 km.<sup>2</sup> including the atrio, outer slope of the central cone and a part of outer slope of the somma, where the vegetation composed mainly of *Reynoutria hachidyoensis* and *Carex Okuboi* is very sparse (Pl. 4). Places where the parent rock is unfavourable for plant colonization remain bare.

## V. DEVELOPMENT OF PLANT COMMUNITIES

From the description outlined above, it may be easily suggested that each of the vegetation types except for artificial forest is a seral successor developing from pioneer stage to climax one. In this chapter the dynamics of vegetation is described with special reference to vegetation structure.

### A. The Sere

The first substratum available for the plant colonization is freshly deposited lava or volcanic ash. But main substratum which is now undergoing active plant invasion is the lava flow of 1778. As soon as the lava is weathered mechanically and sand accumulates to some extent, *C. Okuboi* and *R. hachidyoensis* begin to invade the lava. It is very noticeable here to denote that neither lichens nor mosses play any important role for weathering of the parent rock because no appreciable amount of them can be seen even on the lava flow of 1778. This fact holds naturally good for the lava flow of 1950 and 1951, on which these plants cannot be found at all. Accordingly it appears that many years are needed for weathering of the parent rock, subsequently for the establishment of desert community.

As mentioned before, the desert occupies at present the vast area of the lava flow of 1778 and a part of central cone ejecta. The vegetation is very sparse, having coverage of 10-20 %, as illustrated in Fig. 4. Both *C. Okuboi* and *R. hachidyoensis* have well-developed rhizomes, suggesting instability and xeric

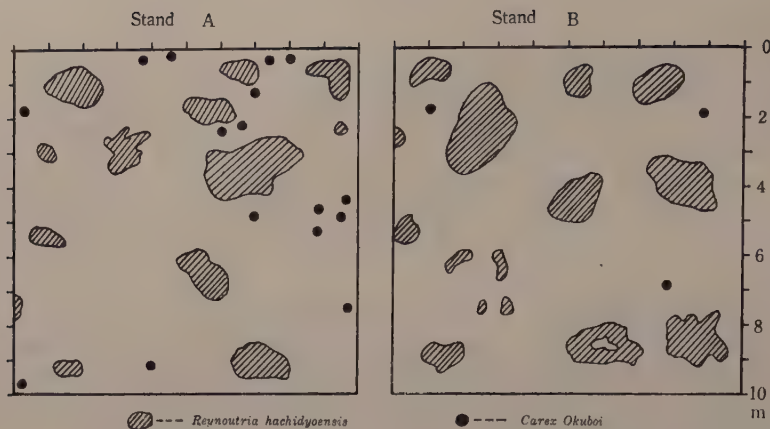


Fig. 4. Diagrammatic presentation of the distribution of herbage colonies in desert.

nature of the sand.

It is a very interesting fact that any herbaceous species other than the above-mentioned two does not invade the desert positively so that grassland vegetation may be composed, in spite of the fact that many species available for the formation of grassland community appear all together elsewhere in the island. These species are, for instance, *Echinochloa Crus-galli*, *Digitaria ciliaris*, *Eleusine indica*, *Miscanthus sinensis*, *Setaria viridis*, *Agropyron semicostatum*, *Agrostis Matsumurae*, *Sporobolus elongatus*, *Achyranthes japonica*, *Desmodium racemosum*, *Lespedeza bicolor*, *Oenothera odorata*, *Artemisia vulgaris*, *Aster Yomena*, *Lactuca laciniata*, *Erigeron linifolius*, *Cirsium* spp. and so on. Many of these species have dissiminules dispersed easily by wind. Accordingly the soil of desert seems too barren and xeric to permit the colonization of these plants.

The first tree invader into the desert is *A. Sieboldiana*, which forms before long spatially dispersed scrub. After *A. Sieboldiana* has established on the barren sands, other tree species such as *W. grandifolia* begin to invade the vegetation and then *Ligustrum pacificum*, *Callicarpa japonica* var. *luxurians*, *Euonymus Sieboldianus*, *Stachyurus praecox* var. *Matsuzakii* and so on. Thus the closed scrub is formed. But the scrub stage is unstable, for the dominants of scrub give way to such deciduous trees as *P. Lannesiana* form. *simpliciflora*, *C. controversa* and *Styrax japonica* var. *iciomotensis*, which form the tree layer of the mixed forest. In parallel with invasion of these deciduous trees, such evergreen broad-leaved shrubs as *Eurya japonica* var. *montana*, *Neolitsea Sieboldii*, *Cammelia japonica*, *Aucuba japonica* and *Cinnamomum japonicum* invade the scrub, all of which, however, form the sub-tree layer of the mixed forest. In such a way, the mixed forest with wide distributing area is built. This forest stage seems to continue for a while until the maturation of structure. But the matured mixed forest has no self-maintaining ability. Supports for this conclusion are provided by the fact that no young trees of dominant deciduous trees can be found under the canopy of matured mixed forest, while those of dominant species detected in the

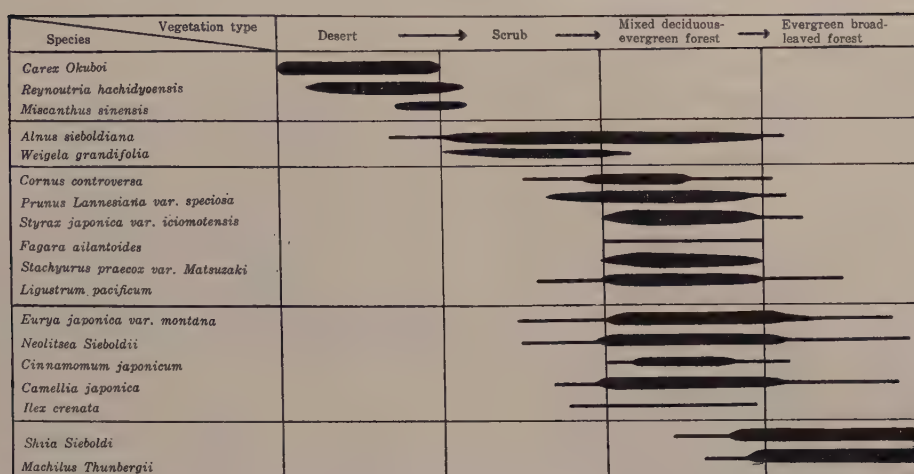


Fig. 5. A schema illustrating the alternations of principal plant species in the normal succession.

evergreen broad-leaved forest can be found abundantly under both canopies of the matured mixed forest and evergreen broad-leaved forest. Thus evergreen broad-leaved forest is the climatic climax which maintains itself indefinitely if left undisturbed. The alternations of principal plant species accompanying the normal succession are summarized schematically in Fig. 5. As a conclusion, one can accept the primary succession with the following sere in this island; bare land → "desert" → scrub → mixed deciduous-evergreen forest → evergreen broad-leaved forest.

### B. Quantitative Data on the Structure of Communities

The broad features of a primary plant succession are reflected in the vegetation structure, of which quantitative data are now briefly presented.

Degree of Covering and Height of the Vegetation:—

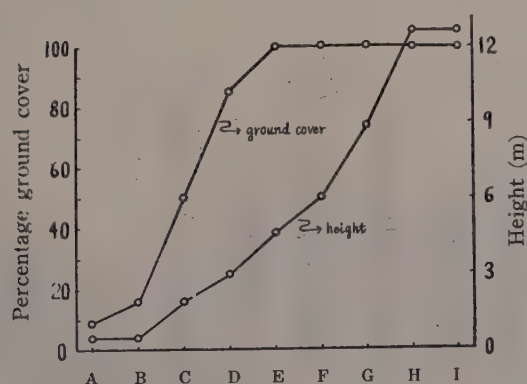


Fig. 6. The changes in degree of covering and height of the vegetation.

The cover degree obtained for dominant layer and the height of the vegetation are illustrated in Fig. 6, where the curves show quite clearly the seral changes in the vegetation structure.

Abundance:— Numbers of tree species and of their individuals having diameter breast high above 2 cm. in unit land area are illustrated in Fig. 7, which shows how these numbers increase rapidly in the early stages and decrease in the later stages of succession.



The reason of decrease in number of individuals and tree species in the later stages may probably be due to competition, for which the author, to his regret, has no available data at present.

**Basal Area:—** The changes in total basal area are shown in Fig. 8. For comparison, in the figure are also presented the data of the warm temperate forest of Mt. Kiyosumi, the Boso Peninsula (see Fig. 1), where *Cyclobalanopsis stenophylla* dominated and that of

the southern part of the Osumi Peninsula, Kyushu [29]; both data were obtained by the present author. From the figure it is easily accepted that the basal area increases with the successional progress of the communities, and that the ever-green broad-leaved forest of this island is never inferior in its richness expressed in basal area to those found in another warm temperate regions of Japan.

**Basal Area Ratio:—** Dominancy diagrams of each forest stand are shown in Fig. 9, where the dominance is represented by basal area ratio. The figure shows clearly how the dominance of alder, a pioneer tree species, gives way to that of another species in the course of succession. Furthermore, it is of interest that *Machilus Thunbergii*, a dominant species of the climax forest, appears in the later stage of the mixed forest.

**Diameter Class Distribution:—** In Fig. 10 are indicated diameter class distributions of each forest stand, where it becomes apparent that the abundance of trees decreases with increase in diameter breast high in every stand, and

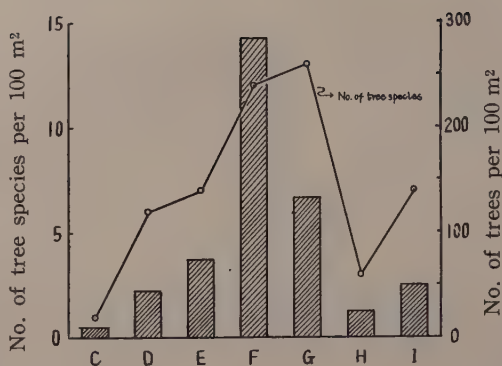


Fig. 7. The changes in number of tree species and their individuals with D.B.H. above 2 cm.

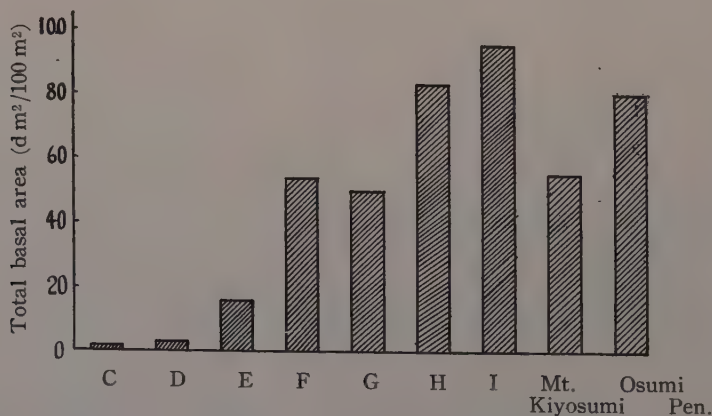


Fig. 8. Total basal area of the various forest stand.

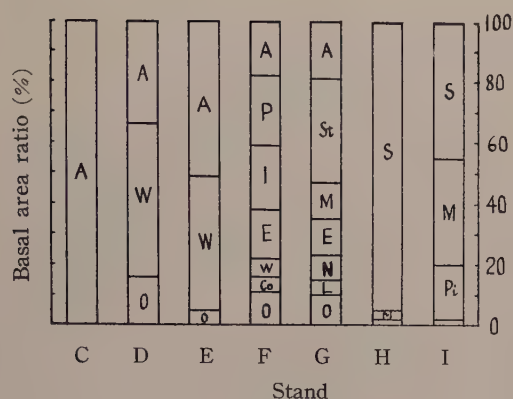


Fig. 9. Dominancy diagrams of each forest stand, where the dominance is represented by basal area ratio.

A: *Alnus Sieboldiana*, W: *Weigela grandifolia*, P: *Prunus Lannesiana* var. *speciosa* form. *simpliciflora*, I: *Ilex crenata*, E: *Eurya japonica* var. *montana*, Co: *Callicarpa japonica* var. *luxurians*, St: *Styrax japonica* var. *iciomotensis*, M: *Machilus Thunbergii*, N: *Neolitsea Sieboldii*, L: *Ligustrum pacificum*, S: *Shiia Sieboldii*, Pt: *Pinus Thunbergii*, O: other species.

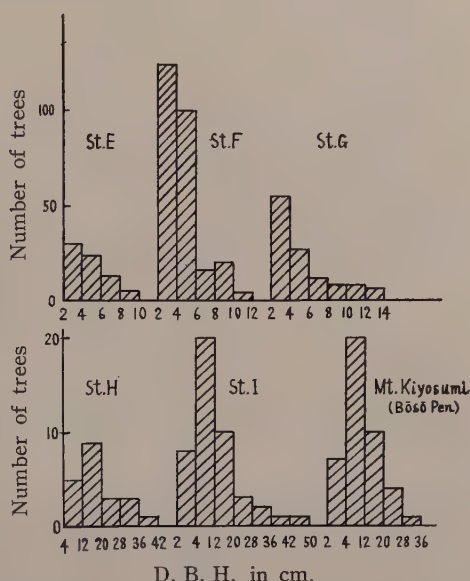


Fig. 10. Diameter class distribution of various forest stands.

mean diameter of each stand increases in accordance with the progress of succession.

From the above-mentioned data it can be concluded that plant succession proceeds in the sequence illustrated in Fig. 5. However, it must be borne in mind that the sequence of different vegetations located at different places does not, of course, represent exactly the past or future course of succession on any single place.

## VI. SOIL FORMATION

Soil formation is one of the most striking phenomena coupled with the reactions of the vegetation. Various aspects of soil formation are now described below.

### A. Development of Soil Profile

On this island can be seen successive stages of soil profiles which develop from an initial undifferentiated lava or volcanic ashes. Theoretically, with the establishment of the "desert" community soil profile must soon or later begin

to differentiate due to the accumulation of dead plant materials. But practically neither litter layer nor humus layer was seen to develop under this community, because a small amount of litter derived from the dead plants is fully swept away by wind action (Tab. 2, Fig. 11). Essential differentiation in soil profile occurs when forest canopy has well developed, under which vigorous accumulation of litter and hence marked development of humus layer can be seen (Tab. 2.) Depth of humus layer increases with the progress in plant succession. But it is a very noteworthy fact that B horizon was not recognizable even under evergreen forest, which is detectable on the oldest volcanic ash accumulated about 1200 years ago (Tab. 2, Fig. 11). Accordingly it appears in this island that more

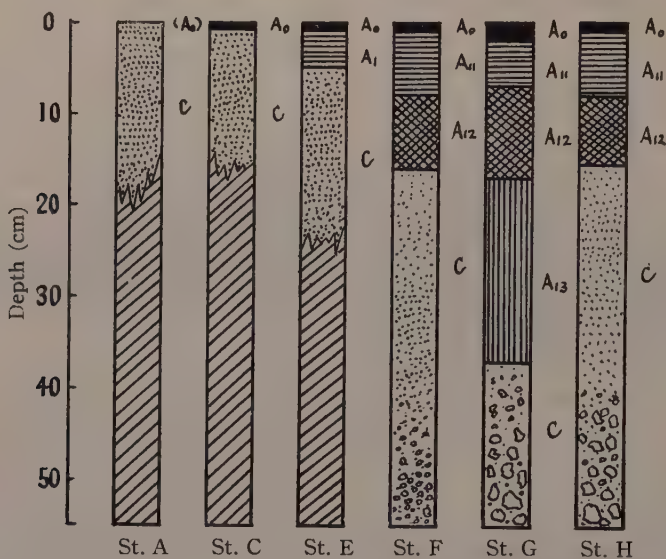


Fig. 11. Schematic presentation of the changes in soil profiles.

TABLE 2.  
Depth of soil profiles and their humus content (ignition loss)

Profile	Stand A (Desert)		Stand E (Scrub)		Stand F (Mixed forest)		Stand G (Mixed forest)		Stand H (Climax forest)	
	Depth (cm)	Humus (%)	Depth (cm)	Humus (%)	Depth (cm)	Humus (%)	Depth (cm)	Humus (%)	Depth (cm)	Humus (%)
A <sub>0</sub>	0	—	1.0	—	0.5	—	2.0	—	0.5	—
A <sub>11</sub>	0	—	4.0	7.49	7.0	18.73	5.0	26.75	7.0	13.41
A <sub>12</sub>	0	—	0	—	7.5	4.15	14.0	14.83	7.5	4.84
A <sub>13</sub>	0	—	0	—	25	2.31	20	4.77	25	—
C	—	0.26	—	0.65	—	—	—	—	—	—

than several thousand years are needed for the maturation of the soil profiles after the first establishment of the climax vegetation. This agrees on the whole with Olson's observation, which showed that about 8000 years had been needed for the maturation of soil profiles of the dune soil near Lake Michigan after the first establishment of climax oak forest [47]. But future course of soil development will, no doubt, lead to the formation of mature brown forest soil under the influence of the warm temperate climate of Oshima, since this soil type has been widely recognized in the warm temperate regions of Japan [29]. A schematic diagram showing the changes in soil profiles during succession is illustrated in Fig. 11. Moreover, depth and humus content of each horizon are summarized in Table 2, where it can be seen that the changes in humus content along the vertical direction in a given stand are similar to the gradients of humus content detectable on the surface layers with various successional ages.

### *B. Changes in Soil Physical Properties*

Only a few analyses were made on the physical properties of soils, but their results may clearly show how they change in parallel with the development of the vegetation. To simplify the expression, many of these changes were arranged here as a function of humus content.

**Apparent Density:**— Apparent density with initial value of 1.7 decreases rapidly at first with increase in humus content and slowly later on (Fig. 12). This curve can be used to transform the data on a dry weight basis to a volume basis if desired.

**Soil Texture:**— As illustrated in Table 3, all soils analyzed were sandy soils with very small amount of clay, the fact may suggest that the significance of clay mineral for the water-holding capacity and cation exchange capacity is negligibly small in these soils. But it is an important fact that fine sand fraction increases as soil develops (cf. Fig. 11).

**Moisture Equivalent:**— Fig. 13 shows how water relations become suitable for plant life with increase in humus content. This fact is well reflected in

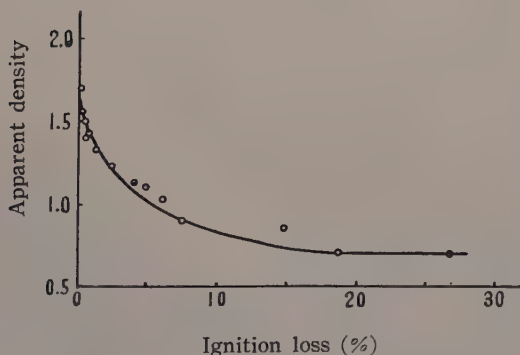


Fig. 12. The changes in apparent density as plotted against the ignition loss of the soil.

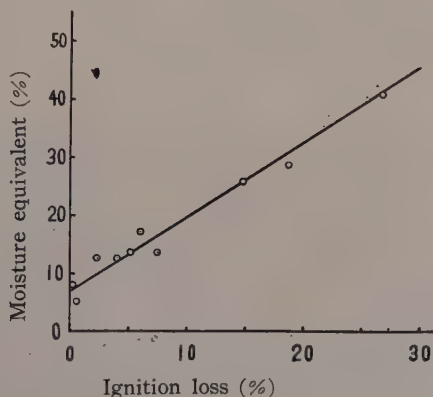


Fig. 13. The increase in moisture equivalent along with the accumulation of humus in the soil.



TABLE 3.

The result of mechanical analyses of various soils. Soil particles were classified according to the nomenclature of Agricultural Society of Japan. Each value in the table is percentage of oven-dried fine soil.

Stand	Profile	Coarse sand (2.0-0.25 mm)	Fine sand (0.25-0.5 mm)	Silt (0.05-0.01 mm)	Clay (<0.01 mm)
Bare land	C	70.8	24.9	1.4	2.9
A	C	73.1	23.4	0.9	2.6
F	A <sub>12</sub>	44.5	37.4	14.4	3.7
F	A <sub>13</sub>	44.7	38.7	12.8	3.8
G	A <sub>12</sub>	25.0	65.4	5.4	4.2
G	A <sub>13</sub>	46.3	38.7	12.9	2.1
I	A <sub>11</sub>	59.0	37.3	2.6	1.1

such properties as moisture condition of habitat, life form of various species and physiognomy of the vegetation. For example, the morphology of *R. hachidyoensis* is very different on desert and scrub, i.e. on the former it is about two times lower in height and several times smaller in leaf-size than on the latter. At any rate, water content of the soil, together with the content of mineral nutrient, is probably a factor controlling the direction and rate of succession in earlier stages.

### C. Changes in Soil Chemical Properties

Soil is the source and reservoir of nutrients needed for all terrestrial green plants. Therefore, it is a fundamental problem to elucidate how this reservoir is built up and how nutrients are accumulated in it.

Soil pH:— Soil pH with initial value of 5.8 undergoes slight changes as the soil develops (Table 4), i.e. the surface layer becomes slightly more acid and lower layer more basic than parent material. Slight acidification may probably be due to the acidity of litter itself, for it appears that humic acids are not abundantly formed under warm climate as that of this island.

TABLE 4.  
Soil pH of various profiles

Bare land	Stand A	Stand D	Stand E	Stand F	Stand G	Stand I
Prof. pH	Prof. pH	Prof. pH	Prof. pH	Prof. pH	Prof. pH	Prof. pH
C 5.8	A <sub>11</sub> 5.8	A <sub>11</sub> 5.8	A <sub>11</sub> 5.8	A <sub>11</sub> 5.6	A <sub>11</sub> 5.2	A <sub>11</sub> 5.8
		C 6.1	C 6.1	A <sub>12</sub> 5.8	A <sub>12</sub> 5.6	A <sub>12</sub> 6.5
				A <sub>13</sub> 6.3	A <sub>13</sub> 6.4	

Nitrogen:— Changes in content of total, ammonium- and nitrate-nitrogen are shown in Fig. 14 A, B and C. Total nitrogen increases linearly with the increase of humus content (Table 6, Fig. 14). The mechanism by which soil

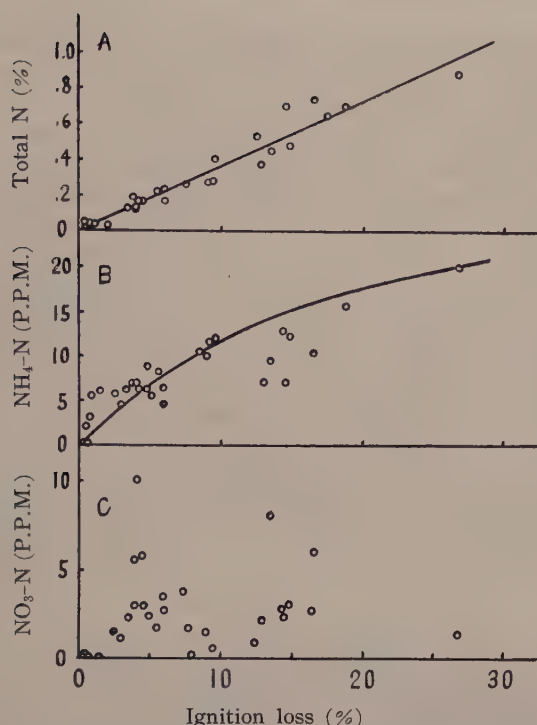


Fig. 14. The changes in total, ammonium- and nitrate-nitrogen of the soil.

nitrogen accumulates will be considered in the following chapter. Ammonium-nitrogen also increases roughly in proportion to humus content, while nitrate-nitrogen shows no correspondence to humus content, the fact suggests that nitrification does not parallel the accumulation of soil organic matter.

Phosphorus:— The results of total and available phosphorus analyses are shown in Fig. 15 A and B, where it will be seen how total phosphorus increases with the increase in humus content. It is very interesting that about 20 p.p.m. of total phosphorus can be found in soils having no organic matter in contrast to total nitrogen (Fig. 14). This fact suggests that phosphorus contained in the parent rock is

absorbed gradually by plants and accumulates in the surface layer through the litter of dead plant materials. But the data of available phosphorus show clearly that it reaches a saturation value of about 4 p.p.m. when humus content reaches about 10%. This result can be readily understood by the assumption that soluble phosphate becomes insoluble by forming such salts as Ca- and Fe-phosphate.

Potassium:— Content of available potassium increases with increase of humus content (Fig. 16).

Calcium:— Quantitatively speaking, the most striking change of various available nutrients can

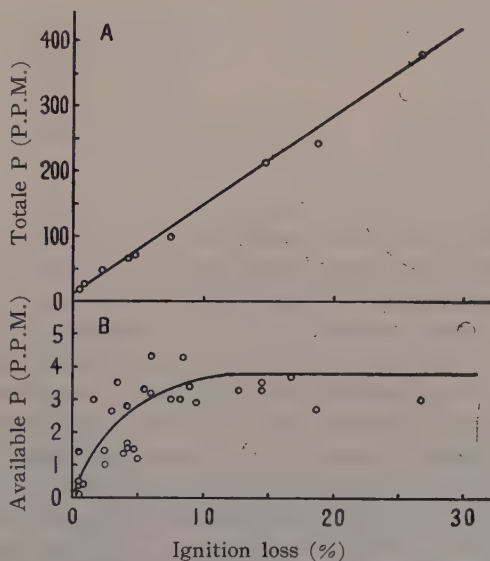


Fig. 15. The changes in total and soluble phosphorus of the soil.

be found on calcium content (Fig. 17). This can be understood by the fact that litter contains calcium most abundantly among various nutrients (Table 5).

Cation Exchange Capacity:— Only a few samples were analyzed to know cation exchange capacity, but it is easily seen from Fig. 18 that the capacity increases along with humus content. Pratt showed experimentally a linear relationship between the amount of organic materials added on an irrigated soil

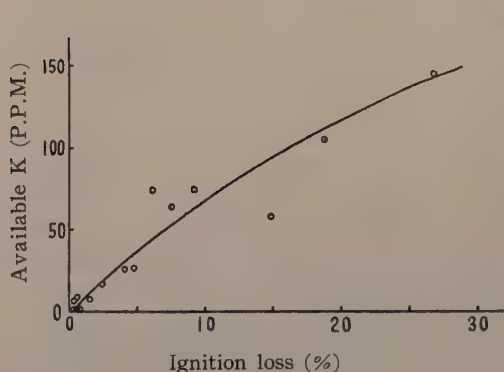


Fig. 16. The changes in available potassium of the soil.

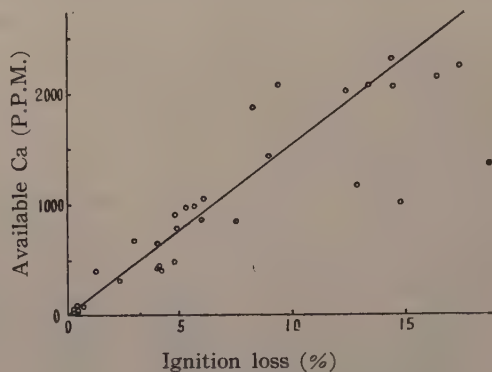


Fig. 17. The changes in available calcium of the soil.

TABLE 5.

Chemical constituents of litter. The data are shown in percentage of dry matter.

Stand \ Mineral	Ash	N	P	K	Ca
F	14.42	1.19	0.09	0.08	2.45
G	11.63	1.53	0.09	0.16	1.55
H	19.02	1.36	0.10	0.13	1.85

and the cation exchange capacity of the soil [52]. As mentioned previously, soils of this island contained minute amount of clay (Table 3), so the cation exchange capacity of these soils is probably determined by humus alone.

#### Amounts of Organic Matter and Mineral Nutrients per Unit

Land Surface:— In the above description, many of the data on the physical and chemical properties of soils were arranged against humus content. From the standpoint of soil-vegetation interactions, however, estimates of the amounts of organic matter and mineral nutrients per unit land surface may be a more satisfactory basis for the better understanding of the dynamic nature of that system. Therefore, these amounts were calculated from the data of Table 2 and Fig. 12, with the assumption that soil volume is constant indifferent of soil water content. The result is summarized in Table 6. In spite of the scarcity of the sampled data, it may be concluded that these estimates show obviously the tendency of the changes in various factors correctly. It can be understood

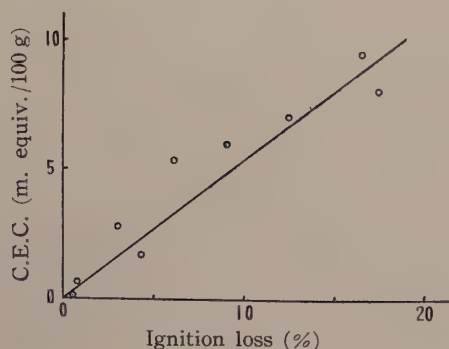


Fig. 18. Cation exchange capacity as a function of humus contents of the soil.

from these data that when a raw parent material first becomes available for plant colonization (stand A), it contains almost no nitrogen compounds, soluble phosphorus, potassium and calcium. With the colonization of pioneer plants on such parent material, these nutrients begin to appear and accumulate progressively. Undoubtedly the pioneer plants on such habitats have low requirements for these nutrients and can accumulate the minute amounts of nutrients which are

given as impurities in rain water or as the weathering products. Moreover, the data show that contents of organic matter and mineral nutrients attain to maximum levels, or, more precisely, to steady state when the sere reached its climax.

TABLE 6.

The amount of organic matter and nutrients contained in the soils of unit land surface (1 m<sup>2</sup>). Organic matter and nutrients contained in the litter layer were omitted from the values illustrated in this table.

Substance \ Stand	A	E	F	G	H
Organic matter (kg)	1.5	4.7	20.0	32.5	28.8
Total N (g)	2	144	515	990	74.3
Available N (g)	0	1.5	4.6	4.1	4.7
Total P (g)	5.7	11.3	47.9	46.0	34.0
Available P (mg)	60	258	699	461	474
Available K (g)	0	2.3	12.1	13.4	13.7
Available Ca (g)	6	54	195	255	311

In order to ascertain whether fertility level determined by chemical analyses of the soil is really reliable in terms of plant growth, soils of surface decimeter were collected on several stands on May, 1959 and brought to laboratory experiment. One liter of the wet soil was filled in the clay pot, and five seeds of sunflower plant were sowed in the soil. Four pots were used for each soil and a half of them were well fertilized with N, P, K and Ca. The pots were kept outdoors for seven weeks with plentiful watering (Pl. 5). As might be expected, native soils were unproductive. The plants without fertilization were smaller on desert soil than on any other soil. But plentiful fertilization resulted in the vigorous growth of the plants on every soil.

Finally, it should be an interesting problem here to give some considerations on the rate of humus accumulation during the succession. But one must be



contented here with a rough estimate, since the changes in humus amounts cannot be plotted here correctly as a function of time. As mentioned before, in the oldest soil of this island about 300 ton of organic matter (dry weight) per hectar has accumulated during the past 1200 years; mean annual increase, therefore, is 250 kg. per hectar. Probably the real annual increase may be greater than 250 kg., because many years are needed for the first accumulation of humus as above-mentioned. Crocker and Major found 500 kg. of annual increase in humus during the succession on a recessional moraine of Alaska they studied [13]. Crocker and Dickson found the value of about 1 ton on another recessional moraine of Alaska [12]. Accordingly, the value of 250 kg. detected here is significantly lower than those estimated by them. This difference may partly be due to large decomposition rate under the influence of warm climate of this island.

## VII. SOIL-VEGETATION INTERACTIONS AND DEVELOPMENT OF THE SYSTEM

In chapter V the author analyzed quantitatively the development of vegetation as the changes of its structural characters, and in the foregoing chapter he described the changes in physical and chemical properties of the soil with the development of the vegetation. Now it has become evident how vegetation and soil develop in parallel way. However, to understand more quantitatively and unifyingly the interdependent development of vegetation and soil the development of vegetation must be analyzed as the changes of its functional characters.

From this point of view the author tried to analyze in this chapter mineral nutrient economy and productivity of the soil-vegetation system, although such a trial in terrestrial communities, particularly in forest communities, has scarcely been undertaken. But the necessity of the study along this line has been pointed out by Malmer and Sjörs [38], Malmer [37] and the present author [64].

### A. *Mineral Nutrient Economy of Soil-Vegetation System*

Estimates of leaf amount and standing crop:— Leaf amount and standing crop of desert community was estimated according to the method described in chapter III. Calculated per hectar, leaf amount was 130 kg. on stand A and 400 kg. on stand B. These values correspond to leaf area of 0.2 and 0.7 hectar respectively. The standing crops were 900 and 1400 kg. on stands A and B, respectively. Of these standing crops, aerial part (leaf and stem) occupied only 20 to 30 % of the values, showing a well-developed subterranean part.

Regarding forest community estimate of leaf amount was made indirectly in the following way. Ten trees belonging to various species and having different diameter breast high were cut down, and the total leaves were directly measured in weight for each tree in the field. Then, leaf area and dry weight of leaves of each tree were determined using sub-samples of a known amount of leaves. From these data it became evident that a linear relationship was found between total leaf area and stem diameter at breast height in a log-log coordinate system (Fig. 19). This relationship is similar to that already reported by Kittredge and

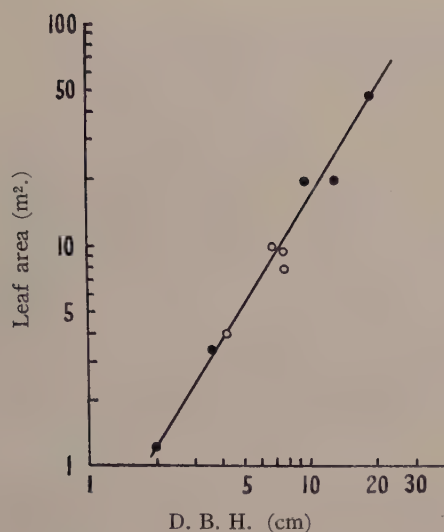


Fig. 19. Straight-line relationship between the total leaf area and D.B.H. of the tree. Black and white circles in the figure show evergreen broad-leaved tree and deciduous one respectively.

trunk and branches and D.B.H., similar to that between leaf area and D.B.H., was found for evergreen broad-leaved trees of the Osumi Peninsula [29]. It has been expressed by the following equation;

$$\log W = 2.34 \log D - 0.87$$

where  $W$  is total dry weight of trunk and branches of tree in kg. and  $D$  is D.B.H. in cm. Accordingly it is possible to calculate total dry weight of trunks and branches per unit land surface from this equation and the data of diameter distribution determined in the field, with an assumption that the equation can be fitted also for the deciduous trees in question. Then standing crop can be calculated as the sum total of leaves, trunks, branches and roots (amount of roots was assessed as one third of the amount of trunks and branches [41]). The dry weight and area of leaves and the standing crop of forest communities thus calculated, together with those of desert community, are shown in Table 7. The data show clearly how the amount of leaves and standing crop increase along with the development of both vegetation and soil. The values of leaf amounts obtained for the mixed forest and evergreen forest were exceedingly larger than those obtained for various deciduous forests [56]. But it is of great importance from the standpoint of plant reaction to the soil through leaf fall that the longevity of leaves is several months for deciduous trees and about one year for evergreen broad-leaved ones, while it is several years for coniferous ones [cf. 42]. Moreover, the value of 420–460 ton detected for the standing crop of evergreen

Sato et al. for coniferous trees [30, 57], and by Morisawa et al. for beech [45], but different in that this lineal relationship can be found among various species in this case, since the workers above-mentioned have dealt with individuals of various size in one species. A similar result to that obtained here has recently ascertained by us for evergreen broad-leaved trees of the Osumi Peninsula [29]. From this figure and diameter distribution measured directly in the field, leaf area of each species per unit land area was calculated. Then leaf dry weight per unit land area was estimated on the basis of area/dry-weight ratio of leaves which was determined for each species. Concerning the amount of trunks, branches and roots per unit land surface, no available data were obtained in the present investigation. But a linear relationship between total dry weight of

TABLE 7.  
Standing crop (dry weight) and leaf area of various stands

Stand	A	B	E	F	G	H	I
Leaves (ton/ha)	0.13	0.4	3	11	8	13	11
Trunks and branches (ton/ha)	—	—	36	125	131	307	339
Roots (ton/ha)	—	—	(12)	(42)	(44)	(102)	(113)
Total (ton/ha)	0.9	1.4	51	178	183	422	463
Leaf area (ha/ha)	0.2	0.7	3.9	13.7	9.9	11.9	11.2

broad-leaved forest is the greatest values which have ever been estimated for various forests [41, 50, 56, 57, 58].

Mineral nutrient economy:—

One of the most interesting problems associated with mineral nutrient economy is to know how differences in soil fertility are reflected in the nutrient concentrations of plants grown under their own habitats. In Table 8 are presented the results of chemical analyses of leaves collected in the mid-summer of 1958. There was no clear correlation between mineral concentrations in leaves and soil fertility as in the reports of Malmer and Sjörs [38] and Malmer [37]. The present data show that mineral concentrations of evergreens are slightly lower than those of other plants. Furthermore, it is very interesting that nitrogen concentration is comparatively high in *A. Sieboldiana*, a pioneer tree invading

TABLE 8.  
The result of chemical analyses of leaves. The values are percentage of dry matter.

Species \ Nutrient	Ash	N	P	K	Ca
<i>Reynoutria hachidyoensis</i>	5.64	1.68	0.17	0.62	0.97
<i>Carex Okuboi</i>	—	1.19	0.07	1.05	0.59
<i>Alnus Sieboldiana</i>	6.49	2.29	0.23	0.46	1.12
<i>Weigela grandifolia</i>	7.85	1.44	0.14	0.64	1.26
<i>Prunus Lannesiana</i> var. <i>speciosa</i>	8.86	2.04	0.17	0.64	2.34
form. <i>simpliciflora</i>					
<i>Styrax japonica</i> var. <i>iciomotensis</i>	5.69	2.22	0.16	0.52	1.38
<i>Callicarpa japonica</i> var. <i>luxurians</i>	10.31	2.98	0.16	1.08	1.04
<i>Shiia Sieboldi</i>	3.91	1.15	0.11	0.62	0.57
<i>Machilus Thunbergii</i>	5.00	1.79	0.19	0.85	0.50
<i>Ilex crenata</i>	5.58	1.49	0.11	0.48	1.22
<i>Eurya japonica</i> var. <i>montana</i>	7.82	1.26	0.10	0.44	1.28
<i>Neolitsea Sieboldii</i>	3.83	1.61	0.13	0.31	0.68
<i>Cinnamomum japonicum</i>	5.38	1.55	0.11	0.50	0.87
<i>Camellia japonica</i>	8.54	1.63	0.15	0.35	1.36

actively the desert, while the soil which bears the tree species contains almost no available nitrogen. The fact can be readily understood when nitrogen fixation by its nodules is taken into consideration [4].

The above mentioned fact indicates that the amount of nutrients needed for the production of a unit dry matter is similar among different plant species grown under different nutritional conditions. Therefore, the reason why each representative plant grows in each habitat may be the differences in ability to absorb nutrients which exist in the habitat in various availability.

Another question is how differences in soil fertility are reflected in mineral nutrient economy of the vegetations, which develop from barren desert to climax forest; i.e. what amounts of mineral nutrients are used annually for the production of dry matter per unit land surface. This phase of ecology is of particular importance to the analysis of vegetation dynamics, though it is a very difficult task to make clear definitely, since the estimate of dry matter production itself is very difficult.

Amount of nutrients absorbed annually by vegetation per unit land surface can be represented roughly by the amount of nutrients contained in the leaves, since the total amount of nutrients absorbed annually is largely used for the production of leaves. In Table 9 are given the values on the amounts of nutrients contained in the leaves, being calculated from the data of Tables 7 and 8. It will be evident that the total amount of absorbed nutrients increases with the progress of succession.

TABLE 9.  
The amounts of nutrients contained in the foliage of stands (kg/ha)

Nutrient \ Stand	A	B	C	D	E	F	G	H	I
N	2.2	6.7	14	11	62	199	166	140	160
P	0.2	0.7	0.8	1.1	6.2	15.5	12.9	14.0	16.0
K	0.8	2.5	1.7	3.0	16	52	40	63	73
Ca	1.3	3.9	2.0	7	37	145	87	67	60

It is valuable here to calculate the ratio of nutrient amounts contained in the leaves (Table 9) to those contained in the soil of unit land surface (Table 6), since it signifies the turn-over rate of nutrients in the soil-vegetation system or the efficiency of nutrient absorption from the soil to utilization of them for the dry matter production. The ratios thus calculated are shown in Table 10. The data show that the ratio of each nutrient is rather constant for various seral vegetations, although there is great difference in the ratios among different nutrients. In other words, each community seems well adapted to each environment with maximum utilization of nutritional resources.

Amount of nutrients absorbed by "desert" community was exceedingly smaller than those by forest communities. This can be easily understood from the fact that parent material which is available for the colonization of pioneer plant contains almost no available nutrients (Table 6). Nitrogen is of special



TABLE 10.  
The ratio of nutrients contained in the leaves to those in the soil

Nutrient \ Stand	A	E	F	G	H
Total N / total N	0.12	0.04	0.04	0.02	0.02
Total N / avail. N	—	4.1	4.3	4.0	3.4
Total P / avail. P	0.3	2.4	2.2	2.8	2.9
K / avail. K	—	0.70	0.43	0.30	0.46
Ca / avail. Ca	0.02	0.07	0.07	0.03	0.02

interest here, since this element is not contained in the parent material. The invasion of pioneer plant on such barren parent material free of nitrogen must be made possible by the supply of nitrogen compound dissolved in rain water. In order to get more quantitative information on this problem, the mineral constituents in rain water, collected at Motomachi (cf. Fig. 1) in March, 1958, were analyzed (Table 11). According to Miyake [40], mean concentrations of ammonium- and nitrate-nitrogen of rain water collected at Tokyo were 0.56 and 0.06 mg. per liter respectively. If the nitrogen concentration of rain water fallen in Oshima is assumed as 0.25 mg. per liter, the amount of nitrogen brought about by annual rainfall of 3000 mm. is 7.5 kg. per hectare. This value is larger than the nitrogen amount absorbed annually by the desert community. Therefore, the growth of the desert community seems to be well guaranteed by im-

TABLE 11.  
Mineral constituents of the rain water collected at Motomachi  
in March, 1958

Mineral	mg/L	Mineral	mg/L
NO <sub>3</sub> -N	0.24	SO <sub>4</sub> -S	3.6
NH <sub>4</sub> -N	0.01	Cl	21.0
PO <sub>4</sub> -P	0.01	Ca + Mg	trace

purities in rain water with respect to nitrogen economy. The fact that the amount of nitrogen absorbed by the scrub is exceedingly larger than that by the desert community may probably be due to nitrogen fixation by nodules of *A. Sieboldiana*, as described before. There was no great difference between the amounts of nutrients absorbed by the mixed forest and the climax forest, although a great difference was observed between their standing crop. This may suggest that mineral nutrient economy plays rather a small part in the succession from the mixed forest to the climax evergreen forest.

### B. Productivity of the Vegetation

As shown in the previous paragraph, soil fertility was not reflected in the mineral concentration of the leaves but in the total amount of nutrients contained in all the leaves of a plant community. In another expression, soil fer-

tility is an important factor determining the amounts of leaves of various seral communities. Therefore, soil fertility must influence principally the total photosynthesis or dry matter production of a plant community by changing the amount of leaves. As the direct measurement of total photosynthesis of a community is difficult, Monsi and Saeki [44] and Saeki [54] have introduced several mathematical equations for the calculation of the dry matter production occurring in nature.

According to Saeki [54], mean total daily net photosynthesis ( $P$ ) of the foliage as a whole of a plant community can be obtained by the following equation,

$$P = \frac{b}{Ka} \ln \frac{(1-m) + KaI_0}{(1-m) + KaI_0 \exp(-KF)} - \bar{r}F$$

where  $a$  and  $b$  are constants which characterize the shape of the curve showing the relation between relative light intensity and daily real photosynthesis,  $m$  is leaf transmissibility,  $K$  is extinction coefficient in the foliage,  $F$  is the leaf area index,  $I_0$  is incident light intensity, and  $\bar{r}$  is the mean respiration rate of all the leaves.

Although no plentiful data are obtained as yet for the calculation of the equation, a rough estimate of productivity (Monsi's surplus production [42]) has been made by using coefficients which are either determined directly in the stand or induced from various papers. The value of  $K$  was determined from the relative light intensity within the communities and their leaf area index,  $F$ , on the basis of Monsi and Saeki's equation [44]. Leaf transmissibility ( $m$ ) was assumed as 5.0 %, because Kasanaga and Monsi [26] showed that many of the leaves of deciduous and evergreen trees had transmissibility of 3.0 to 8.0 %.

Photosynthetic and respiratory activities of the leaves, consequently the values of  $a$ ,  $b$  and  $\bar{r}$ , are changeable to a considerable extent with species and seasons. However, Kusumoto [34, 35], Saeki and Nomoto [55], and Saeki [54] have obtained the mean value of net photosynthesis of 5-6 mg. CO<sub>2</sub>/50 sq. cm./hr. in deciduous and evergreen broad-leaved trees under the condition of light-saturation, normal atmospheric CO<sub>2</sub> and 25°C. The values,  $a$ ,  $b$  and  $\bar{r}$ , seem to be rather constant during growing season irrespective of plant species. Therefore, the values obtained in *Zelkova serrata* by Saeki [54] were adopted here as a basis of the calculation of the production during growing season (assumed as about 200 days). Production by evergreen trees during winter (assumed as 165 days) was calculated on the assumption that maximum photosynthesis and the rate of respiration during winter are both one third of those during summer, according to low photosynthetic activity of evergreens in winter reported by Wilson, Kusumoto, and Saeki and Nomoto [34, 35, 55, 67]. Furthermore, incident light intensity ( $I_0$ ) during summer and winter was assumed as 100 and 60 % of mean light intensity for four months in summer measured at Tokyo (cf. Saeki [54]), respectively.

The surplus productions in ton/ha/year of the main stands estimated by the above equation are as follows; stand A (desert)=1.2, stand E (scrub)=43.6, stand G (mixed forest)=61.0, and stand H (evergreen broad-leaved forest)=64.5. From these values, it may well be accepted that the productivity of the vegetation

increases along with the progress in primary succession. The difference in

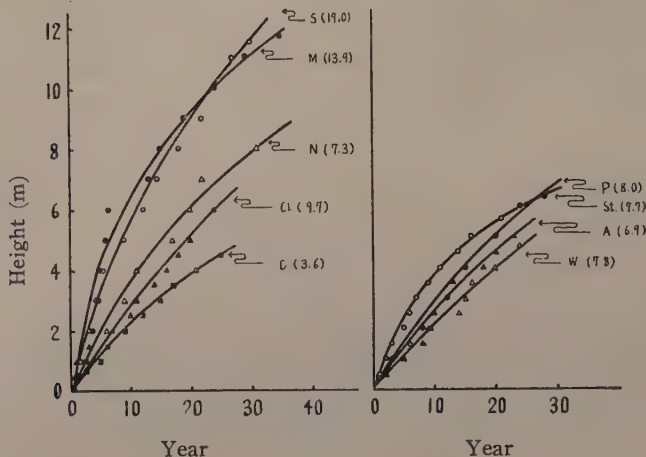


Fig. 20. Growth curves of various trees reconstructed from the stem-analysis. Each letter of S, M, N, etc. represents each species as had been shown in Fig. 9. C: *Camellia japonica*. Number in the parenthesis represents D.B.H. (cm) of that tree.

productivity among various stands is well reflected in the growth rate of the constituent trees, which was reconstructed from the results of stem-analysis (Fig. 20). Most of the trees used for this purpose were the same as those used for the determination of leaf amount. Growth rate of *Machilus Thunbergii* and *Shiia Sieboldi*, the dominants of the climax forest, was larger than that of other trees. Moreover, the growth rate of *Neolitsea Sieboldii*, *Cinnamomum japonicum* and *Camellia japonica*, all of which were constituents of the sub-tree layer of the mixed forest, was similar to those of the deciduous trees which grew as the dominants of scrub and the mixed forest community.

## VIII. DISCUSSION

Various types of vegetations are recognized in the volcanic island of Oshima with various chronological volcanic deposits. Each of these types but artificial forest is quite obviously a seral successor developing from pioneer stage to climax stage.

There have been presented many data to show the changes in species composition, structure of the community and economics of mineral nutrients, and the change of the habitat factors during the succession. The ecological implications of these results need now to be evaluated synthetically.

### A. Development of Vegetation

When the successional relations with respect to floristic composition and

structure of vegetation are examined quantitatively, it is an important problem to determine what quality of vegetation one should select as a criterion of the development of vegetation. Curtis and McIntosh stated that any criterion expressing the changes of dominance is well to be adopted, since the dominance of pioneer species decreases and that of climax species increases as the vegetation develops [14]. They expressed the relative dominance of each species by the importance value (relative frequency+relative density+relative coverage). But when a succession involves different vegetation types, say, for instance, desert, grassland and forest, the application of such a criterion seems to be often difficult. Therefore, in the present investigation various criteria, i.e. cover degree, height of vegetation, number of individuals and species, total basal area, basal area ratio, etc. were adopted.

As had been described before, lichens and mosses seemed to play no significant role in plant succession and soil formation in rocky places of Oshima, although the roles of them have often been emphasized in textbooks of ecology [66]. Cooper and Rudolph discussed this problem and concluded that "the importance of lichens has been exaggerated, and the developmental story of the vegetation has been over-simplified" [10].

Next, it seems an important fact that so-called herbage stage was not recognizable as a seral stage, though the available flora to comprise grassland community was found on the island. All of the results of recent investigations by Dickson and Crocker, Crocker and Major, Crocker and Dickson, Olson and Keay have shown the same fact [12, 13, 16, 27, 47]. These authors were concerned with various parent materials such as recessional moraine, mud-flow, sand dune, lava flow, etc.. Therefore, the appearance of herbage stage at the regions with plentiful rainfall must be confined to the succession under the influence of special factors such as human agency, fire, etc..

The climax vegetation of this island was concluded to be evergreen broad-leaved forest (warm temperate forest). Supports for this conclusion are given by various information. Basing on the warmth index, the total of mean monthly temperature above 5°C, Kira showed clearly that any region of Eastern Asia with the index above 100°C and plentiful precipitation results in the establishment of evergreen broad-leaved forest as the climatic climax [28]. Calculated from Table 1, the index of this island is 118°C; this suggests that the climate of Oshima will bring about evergreen broad-leaved forest as the climax. Suzuki and Hatiya found the same climax at the Izu Peninsula lower than 600 m. in elevation [61]. In addition, Suzuki and Wada recognized also the same climax at the southern part of the Boso Peninsula [62]. The climate of these peninsulas, as far as the lower part than 600 m. in elevation is concerned, is rather similar to that of Oshima. These facts may support above conclusion as well.

### *B. Soil Formation*

According to the view of Jenny and his co-workers [11, 25, 36], any property of a soil is affected by such independent variables as the climatic (*cl*), biotic (*o*), parent material (*p*), topographic (*r*), and time (*t*) factors. This view is correct in a broad sense. As far as this island is concerned, other factors than time



factor are comparatively "constant". Consequently any property of a soil ( $s$ ) is a function of time factor, i.e.,

$$s = f(\text{time})_{a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, x, y, z}$$

This factorial equation implies that differences in soil properties detectable on different places of this island can be comparable each other as a chronosequence. In this respect was found the uniqueness of this island as a research field.

As pointed out by Braun-Blanquet's school [19], each plant community has its own soil profile. That was the case in Oshima, and a close correlation was found between vegetational development and soil formation. But it must be borne in mind that differentiation of soil profile was continuous as the forest stands of various types formed a continuum of vegetation [14]. It was a remarkable fact that mature soil profile was not observed even after the first establishment of climax forest. Here the soil developed only A and C horizons but not B horizon. The differentiation of B horizon needs more time than the first establishment of the climax vegetation does. But the future course of succession will obviously bring about "brown forest soil" with A, B and C horizons.

### C. Soil-Vegetation Interactions

A better approach to the understanding of soil-vegetation interactions on quantitative basis seemed to become possible when the mineral nutrient economy and productivity of the system are analyzed, since the basis on which the life of community is supported is the economy of various substances such as water, nutrients, organic matter, etc. which are all the essential constituents of the living organisms. Although the data on nutrient economy and productivity were rough, they showed correctly the dynamic nature of edapho-vegetational relations.

### D. A Possible Explanation of the Causes of Vegetation Replacement

A possible explanation of the causes of plant succession is now briefly described, though the problem seems too complex to be explained completely.

Freshly deposited lava is unfavorable for the colonization of any plant species because of its barren rocky mass, but when the parent material is weathered mechanically by climatic factor to some extent and sand accumulates, a few herbaceous species can form a spatially dispersed community. The reason of successful establishment of the species comprising desert community is attributed chiefly to their high tolerances to low fertility level, and soil moisture content and to

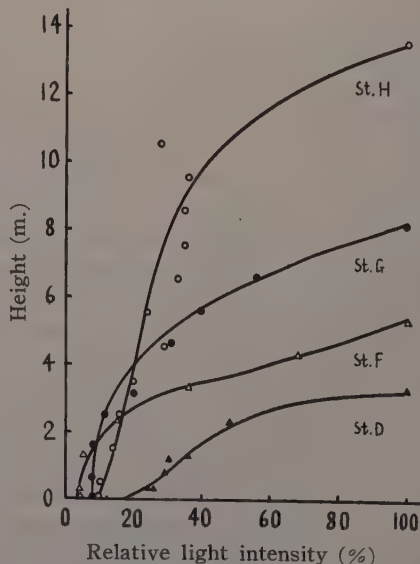


Fig. 21.

instability of the sand. As the mechanical weathering of the rock proceeds, alder, pioneer tree species, begins to invade the desert community on account of its nitrogen fixing root nodules, and before long it forms the open scrub. Then alder makes the soil fertile. As a result other deciduous trees can colonize successfully under the open canopy of alder trees. Thus the deciduous forest is formed. But dominants of the deciduous forest have no self-maintaining ability, since they are suntrees and cannot reproduce their seedlings under low light intensity of mature deciduous forest (see Fig. 21). On the other hand, dominants of the climax forest, i.e. *Shiia Sieboldi* and *Machilus Thunbergii*, are shade-tolerant, so they can compete successfully with deciduous trees. As a result evergreens can form the climax forest, which maintains itself perpetually if left undisturbed.

### SUMMARY

A quantitative analysis of the correlation between primary succession and soil development was made in the volcanic island of Oshima, Izu, Japan, with special reference to the role of edaphic factors in the course of plant succession. Parent materials with different ages comprise the surface layers of the land, and they provide an excellent example for the study of primary succession. The main results obtained are as follows:

1. The typical primary succession in this island proceeds in the following sere: bare land → rock desert (dominated by *Reynoutria hachidyoensis* and *Carex Okuboi*) → scrub (dominated by *Alnus Sieboldiana* and *Weigela grandifolia*) → mixed broad-leaved forest (dominated by *A. Sieboldiana*, *Prunus Lannesiana* form. *simpliciflora*, *Cornus controversa*, etc.) → climax broad-leaved evergreen forest (dominated by *Shiia Sieboldi* and *Machilus Thunbergii*). Concerning the island studied here, the role of mosses and lichens was negligible in the first stage of the xeroarch succession, and the establishment of grassland community as a seral stage was not recognized.

2. The changes in floristic composition and structure of vegetation during the succession were analysed by the quadrat method. Number of constituent species of the vegetation reached a maximum in the mixed forest stage, and decreased rather in the climax forest. Height, cover degree and total basal area increased along with the succession of the forest vegetation. Replacement of dominant species was illustrated in terms of basal area ratio of the constituent tree species.

3. Soil formation was studied by tracing the changes in physical and chemical properties of the soil. Practically, differentiation of soil profile began with the establishment of scrub. Humus accumulated in the soil as the forest vegetation developed, but the true B horizon was not observed even under the climax forest on the oldest (about 1200 years old) volcanic deposit.

4. Total nitrogen, ammonium-nitrogen, total phosphorus, soluble calcium, soluble potassium, cation exchange capacity and moisture equivalent of the soils increased in proportion to humus content. Soluble phosphorus and nitrate-nitrogen contents, however, had no dependency to the humus content. Amount of

nutrients and organic matter per unit land surface increased along with the succession of the vegetation until they reached a steady state in the climax stage.

5. Functional aspects, particularly nutrient economy, standing crop and productivity of the soil-vegetation system were correlated with its development. Differences in the fertility levels of soils were not reflected in the mineral concentration of the plant tissues, but in the total amounts of nutrients absorbed by the plant communities. Constant ratios were observed between the nutrient amount in the soil and that in the foliage.

6. A rectilinear relation was found in log-log coordinate between the leaf area and the diameter breast high irrespective of tree species. The standing crops (ton dry weight per ha) roughly estimated were in desert community 1.2, in scrub 51, in mixed forest 181 and in broad-leaved evergreen forest 443 ton.

7. With several reasonable assumptions, the surplus production in ton/ha/year of each seral community was calculated as follows; volcanic desert 1.2, scrub 43.6, mixed forest 61.0, and broad-leaved evergreen forest 64.5. This calculation was well supported by the growth-analysis of various trees based upon stem-analysis.

8. A possible explanation of the causes of plant succession was made. Edaphic factors, especially soil fertility and soil moisture, seemed to play a decisive role in the early stage of succession, i.e. succession from the bare land to the rock desert, and from the latter to the scrub and mixed forest, while the succession from the mixed forest to the climax broad-leaved forest seemed to be directed by light factor.

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## EXPLANATION OF PLATE I.

- Photo. 1. View of Mt. Mihara, the central cone of this volcanic island, from north-west side of the summit of the somma.
- Photo. 2. Mixed deciduous-evergreen broad-leaved forest. The stand is dominated by deciduous trees.
- Photo. 3. Climax forest comprising mainly *Shima Sieboldi* and *Machilus Thunbergii*.
- Photo. 4. Desert community, which is very sparse.
- Photo. 5. The appearance of sunflower plants grown on fertilized (back) and non-fertilized (front) native soils. The pots represented as "Cult" are those contained the soil of cultivated land in Oshima.



## STUDIES ON THE PHOTOSYNTHESIS AND PRODUCTION OF DRY MATTER IN THE COMMUNITY OF RICE PLANTS

TOMOSHIRO TAKEDA<sup>1</sup>

### INTRODUCTION

The process of photosynthesis involves many simultaneous reactions. In their studies, much of the experimental works have been done on the simplest systems, particularly on the unicellular plants in the Warburg apparatus, which has permitted rigid control of the experimental conditions, or simple aquatic plants. But investigations of the higher plants usually had been confined to the observations of the leaves, either attached or detached.

From the plant physiological point of view, there is no doubt that the information obtained from the studies of the simpler systems and the leaves is very valuable. However, from the agronomical point of view, which regards the individual as the minimum unit of the production, these data can not be applicable to the field problems. On the other hand, several experiments on the relationship between light intensity and photosynthesis of rice plant have been reported recently. Yamada et al [11] found that photosynthesis of detached rice leaves show light saturation point at the light intensity of 40-50 Klux under normal atmospheric carbon dioxide concentration and temperature range of 18-33°C. Matsushima et al [1], measuring photosynthesis of intact rice plants of pot culture, recognized light saturation occurs at about 0.6 cal./cm<sup>2</sup>/min. of solar radiation, and they [2] considered that this value seems to be not affected by the growth stage, by the differences in variety of rice, by the quantity of fertilizer and by the temperature. In contrast, the author and his colleague [8, 9] studying with rice plant growing in pot, reported that the light saturation point is not constant, but it varies with the growth stages or with spacing of seedlings. These results are all obtained from the experiments on the detached leaves or isolated individuals. However, the rice plants growing on the field are consisting of a community, in which complicated interference and competition between individuals prevail. Therefore, the rice plants as the members of the community might behave in quite a different manner with regard to, at least, the relationship between photosynthesis and light. Then, here questions as to the intensity of photosynthesis by the population of rice plants consisted of a large amount of leaves as a whole, and the effect of environmental factors on it, come on the way. But, the data obtained hitherto concerning the photosynthesis of leaves or isolated plant supply no information about the photosynthesis of the rice community under natural conditions.

On the other hand, since most of the dry weight of the plant is derived from the products of photosynthesis, yield data may be regarded as measuring this quantity, but yield data alone supply no information about the relative

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amounts of respiration and photosynthesis, or the detailed effect of environmental factors. Comprehensive studies on the direct relationship between photosynthesis and production of matter in complete systems of higher plants growing under field conditions have not been carried out, though this would be desirable for itself as well as for agricultural stand point.

In order to understand the photosynthesis in connection with the yield productivity and to obtain information useful to practical rice culture, the author has attempted a study on the photosynthesis of the rice plant under community conditions to make clear the relationship between photosynthesis and production of matter.

### MATERIALS AND METHODS

Seedlings, variety Norin No. 29 (medium maturing), grown in nursery bed were transplanted to the specially designed pots made of galvanized-iron plate (13 cm in diameter, 30 cm in height). Arranging these pots in the order of 23 cm×23 cm, an artificial community was formed. Each community consisted of 225 pots.

TABLE 1  
Experimental design (amount of the nitrogen basal fertilization and date of application of nitrogen top-dressing)

(Plot)	30/VI	30/VII	10/VIII	20/VIII	30/VIII	30/X	(Total amount) g. per pot	Kan* per Tan**
A	1.6						1.6	8
B	0.8						0.8	4
C	0.4		0.4				0.8	4
D	0.6						0.6	3
E	0.4						0.4	2
F	0.2	0.2					0.4	2
G	0.2		0.2				0.4	2
H	0.2			0.2			0.4	2
I	0.2				0.2		0.4	2
J	0.3		0.1				0.4	2
K	0.2						0.2	1
L	0.1		0.1				0.2	1
Transplanting		Max. tillering		Heading		Maturing		
		Young ear formation						

Note: \*Japanese unit of weight (1 Kan=3.75 Kg)

\*\*Japanese unit of field area (1 Tan=991m<sup>2</sup>)

Concerning P<sub>2</sub>O<sub>5</sub> and K<sub>2</sub>O, the same amounts were supplied to all plots as a basal fertilizer, 1.4 gr/pot, 1.0 gr/pot, respectively.

Table 1 presents the experimental design. As shown in the table, the plants belonging to each plot were grown under various nitrogen levels differing in the amount of fertilizer and the time of top-dressing. Plots A, B, D, E and K were supplied with nitrogen of different quantity as basal fertilization, plots E, F, G,

H and I were equal in total amount of nitrogen supply, and the ratio of the basal to the top-dressing was 1:1, but they differed in the time of application. Plots E, J and G were equal in total amount of nitrogen supply and in the time of top-dressing, however, they differed from each other in the ratio of the basal and the top-dressing, such as 4:0, 3:1, 2:2, respectively. Plots C, G and L were equal in the ratio and the time of top-dressing, but they differed in total amount of nitrogen-supply. By comparing these plots with B, E, K the differences in the effect of the top-dressing at heading stage induced by the total amount of nitrogen supplied could be detected.

About 10 plants of each plot were sampled, except the plants in the 2 rows at the border of the community, at interval 10 days. After sampling, total leaf area of each plot was measured immediately by means of a usual photographic-paper method. Then, drying samples in the oven, the dry weights of each part of the plant were measured, and then prepared for chemical analysis.

On the other hand, photosynthesis and respiration were measured at arbitrary intervals.

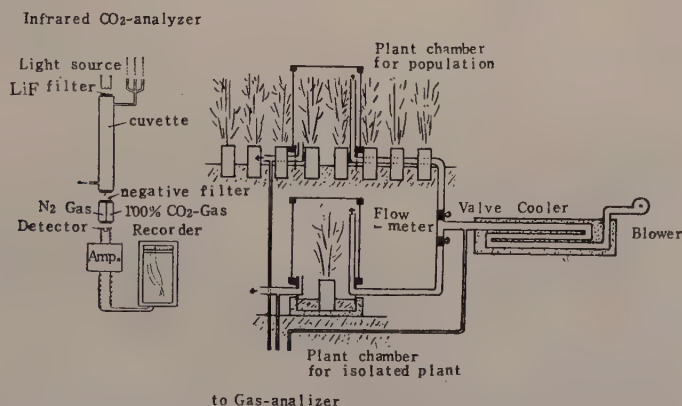


Fig. 1. Apparatus for measuring photosynthesis.

A diagram of the apparatus used in this experiment is shown in Fig. 1, which illustrates two main parts of this equipment, plant chamber provided with a blower and flow-meter, and negative type infrared CO<sub>2</sub> analyzer.

The plant chamber made of wooden frame work and glass plate is set on the chamber acceptor, which is provided with inlet pipe near the ground level of the one side and outlet pipe at the other side, being capable of covering usually four hills of the plants at the artificial community. At the beginning of the measurement, the chamber-accepter is set, and inlet pipe is connected to the blower and flow-meter with plastic tubing, and then the chamber acceptor is covered by the plant chamber. These are made so carefully that the natural situation of the plants and the neighbouring plants is not disturbed. As the blower is driven, fresh air is supplied continuously to the chamber through the air inlet. Rate of air flow is shown by the reading of flow-meter, and is adjustable by the stop-valves. Sample air is taken by the motor-driven piston from both fresh air and the air coming from the chamber, and sent into the gas

analyzer, the operation principle of which has been described in a previous paper [10]. The amount of carbon-dioxide absorbed by the plants can be calculated by the difference in carbon-dioxide content between those two air samples and total amount of the air supplied to the plants. The rate of the air flow into the chamber is regulated so as to keep the depression of  $\text{CO}_2$  content of the air within 10%. The over-rising of temperature within the chamber is prevented both by cooling the supplied air by means of refrigerator and by pouring water over the plant chamber, and thus the temperature is kept within  $2-3^\circ\text{C}$  over the natural one.

Light intensity is measured mainly by Mazda Luxmeter No. 5, and for horizontal radiation intensity the Robizsche's pyrheliometer is used.

## RESULTS AND DISCUSSION

### I Changes in the factors constituting dry mater productivity in the community of rice plants with their growth

#### (1) Changes in respiratory ability of the plant (R) with the growth stages.

The changes in respiratory ability of each plant affected by the amount of basal nitrogen and top-dressing are shown in Fig. 2. As in this experiment the respiration rates are measured under natural conditions, in order to make the "respiratory ability" comparable to each other, the values have been converted into respiration rate under the temperature of  $30^\circ\text{C}$  by means of the temperature coefficient of respiration ( $Q_{10}=2.0$ ), which are illustrated in the figure.

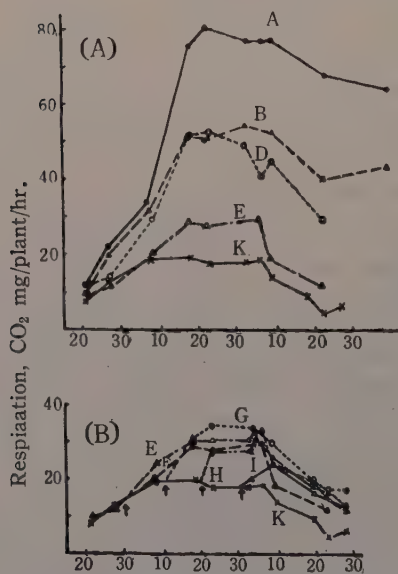


Fig. 2. Changes in respiratory ability with advancement of growth.

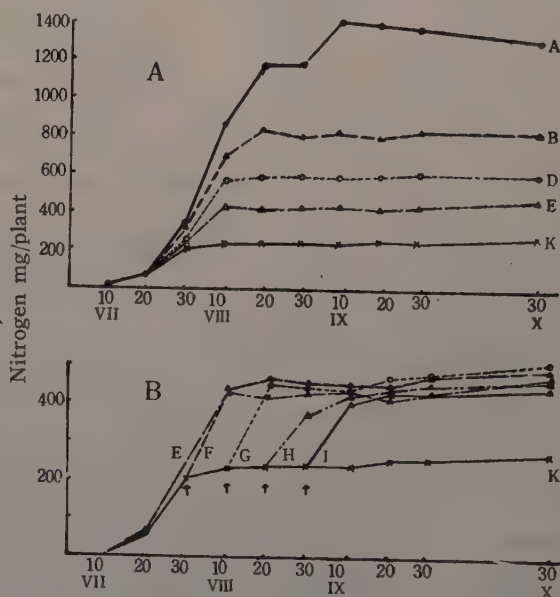


Fig. 3. Changes in nitrogen content of the plant with advancement of growth.

In general, in early growth period respiratory ability of the plant is smaller, with advancement of growth it increases gradually and attains its maximum at the stage from booting to heading, after then decreases steadily during ripening. The higher the level of basal fertilization, the larger the rate of respiration. Nitrogen top-dressing brings about the increase of respiration rate, which attains always the same level, regardless of the time of its application. Comparing such changes in  $R$  with that of nitrogen contents per plant (Fig. 3), at least until about heading stage, a considerable parallelism can be observed between them. Thus, the relationship between them was examined. As shown in Fig. 4, a comparatively high correlation exists between them except for the ripening stage. The relationship after heading forms another correlation group, and the former always takes its position under the latter. The results may be attributed to the fact that as the ripening proceeds the amount of stored protein in the grains increases, and it results in a decrease in the amount of the "living nitrogen." From the above observed fact, it may be considered that the changes in  $R$  is due approximately to the amount of the activated nitrogen compounds.

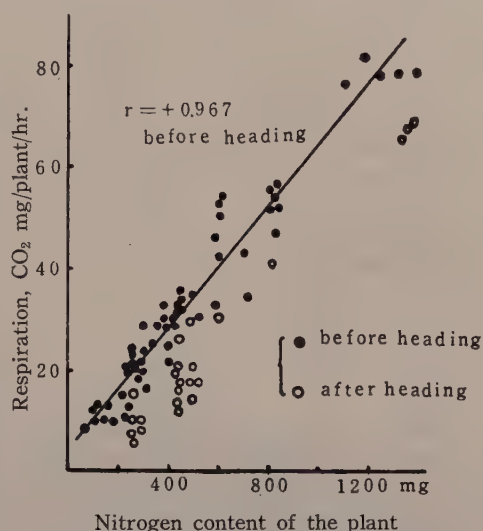


Fig. 4. Relationship between respiratory ability and nitrogen content of the plant.

(2) Changes in photosynthetic capacity of the plant ( $P_0$ ) under isolated conditions with the growth stage.

The individual pot picked up from the community was set in the assimilation chamber under isolated conditions, and the photosynthetic capacity of the plant was measured under the full-sunlight of midday. As this value represents the full photosynthetic capacity possessed by the plant [9] [10], the author proposes here to call it the "photosynthetic capacity of the plant" ( $P_0$ ).

Changes of  $P_0$  in each plot with growth are shown in Fig. 5. As shown in the figure, alike the case of respiration,  $P_0$  increases with advancement of growth, and attains its maximum, and after then falls down. However, the stage at which the maximum  $P_0$  is attained is earlier than in the case of respiration. The higher the level of basal fertilization, the larger the  $P_0$ . Heavy fertilization delays the maximum stage of  $P_0$ . Nitrogen top-dressing always brings about considerable increase of  $P_0$ , regardless of the time of its application. The effect of top-dressing on  $P_0$  decreases with the delaying of its time of application. It is remarkable that the effect on  $P_0$  decreases progressively with advancement of growth especially after the stage of young panicle formation. This point differs sharply from the case of respiration, in which its ability attains always the same level, regardless of the time of application.



Now,  $P_0$  can be divided into two elements; leaf area ( $S$ ), and photosynthetic ability per unit leaf area ( $p_0$ ). Here, the author intends to investigate these two elements separately.

(a) Changes in total leaf area ( $S$ ) with the growth stage.

Total leaf area of an individual plant of each plot shows the changes as shown in Fig. 6. In general,  $S$  is smaller in early growth period, after then

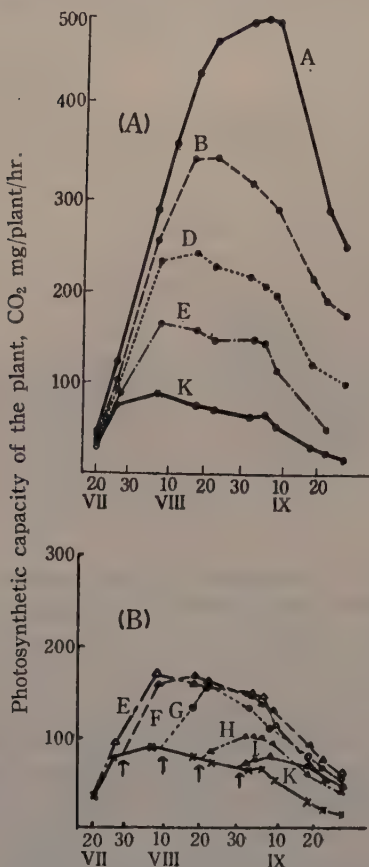


Fig. 5. Changes in photosynthetic capacity of the plant with advancement of growth.

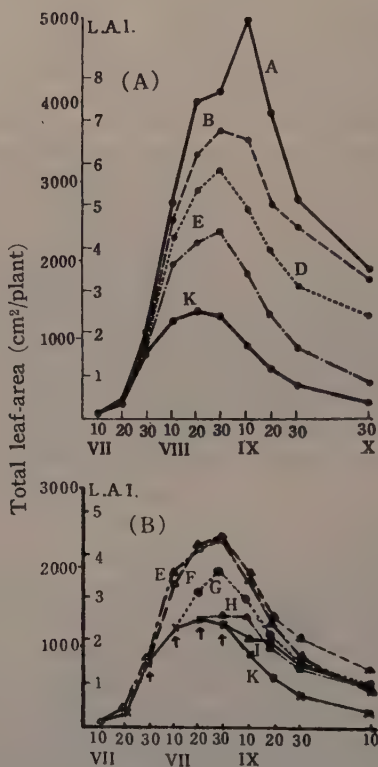


Fig. 6. Changes in total leaf area with advancement of growth.

increases gradually with advancement of growth, and attains its maximum at the stage from booting (about 2 weeks before heading) to the heading, and thereafter falls down with advancement of growth.

The higher the level of basal fertilizing, the larger the total leaf area. In early period of growth the difference of  $S$  brought about by the amount of basal fertilization cannot be distinguished, however it becomes clear gradually with advancement of growth. And further, it can be found that the maximum stage of  $S$  is delayed by increasing fertilization. The larger total leaf area of the

plots supplied with higher nitrogenous basal fertilizer comes from the fact that under heavily fertilized conditions the growth rate itself becomes larger, and the ratio of the amount of leaf blade to the whole plant becomes larger progressively. The delaying of the maximum stage of  $S$  comes from the fact that the time of withering up and the procedure of the withering up of the lower leaf are delayed by the heavy fertilization.

Nitrogen top-dressing always brings about considerable increase in  $S$ , regardless of the time of its application. However, the efficiency of top-dressing decreases progressively, with advancement of growth after the stage of young panicle formation. The causal relationship of above fact can be considered as follows: The nitrogen absorbed during the stage of tillering affects not only the number and size of the leaves, but also withering up. When the nitrogen is absorbed after the stage of young panicle formation, it affects only the size of individual leaves and the withering up. However after emergence of flag leaf nitrogen absorption only prevents the withering up of the leaves already formed.

(b) Changes in photosynthetic ability on leaf area basis ( $p_0$ ) with the growth stage.

Dividing photosynthetic capacity of the plant  $P_0$  by the leaf area  $S$ , the "photosynthetic ability on leaf area basis" can be obtained. Strictly speaking, the photosynthetic rate both of the leaf blades and of the other organs such as leaf sheaths and panicles, are together contained in the  $P_0$ . Accordingly it may be expected that the  $p_0$  obtained by such a way is somewhat larger than the true  $p_0$ . However, it has been proved by the other experiment [7] that the magnitude of the photosynthesis of such organs are negligible.

The changes of  $p_0$  with growth stages are shown in Fig. 7. In general,  $p_0$  attains its maximum at about the stage of maximum tillering, after then it falls down progressively as the growth proceeds. From booting through the heading stages the decreasing trend somewhat declines, and at heading stage a short peak or standstill is observed in some of the plots, and thereafter  $p_0$  again decreases gradually. As regards the magnitude of short peak, it seems that the lower the level of basal fertilization the larger the peak becomes.

And the higher the level of basal fertilization, the larger the  $p_0$  becomes. Nitrogen top-dressing always brings about considerable increase in  $p_0$ , regardless of the time of its application. However, comparing its effects upon  $p_0$  with that upon  $S$ , it is observed that

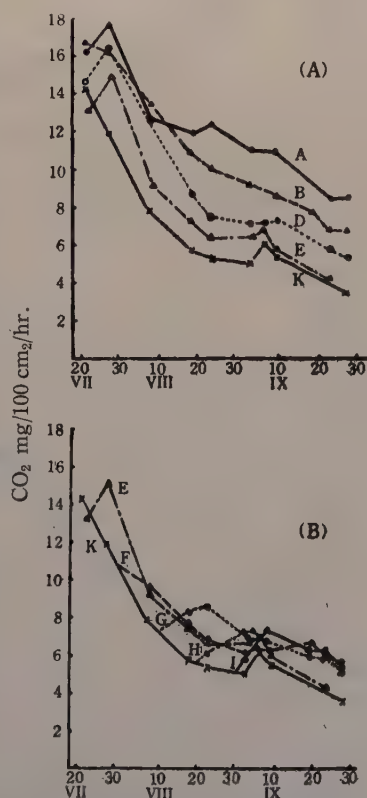


Fig. 7. Changes in photosynthetic ability on unit leaf-area basis.

the former are rather smaller than the latter.

Comparing the changes of  $p_0$  with the variations of nitrogen contents of leaf blade (Fig. 8), an approximate parallelism was assumed. Thus, a further examination was conducted regarding this problem. As shown in Fig. 9, a very high correlation was observed between them. These results approximately agree with the observation of Murata et al [8]. From this fact, it can be said that the  $p_0$  is

determined mainly by the nitrogen contents in leaf blade.

Considering the changes of  $p_0$  and  $S$ , it is clear that the effect of nitrogen top-dressing on  $P_0$  after the stage of young panicle formation decreases gradually, because in each plot, after this stage, the sufficient expansion of leaf area can not be obtained by top dressing.

(3) Changes in field photosynthetic activity of the plant ( $P$ ) as a member of the community with the growth.

Fig. 10 shows the relationship between light intensity and photosynthetic rate of an individual plant under community conditions determined at various stages of growth. It is clear from the figure shown that until 3 weeks after transplanting of the plants, i.e. the stage of tillering, the apparent light saturation points lies between 40 and 50 Klux.

This indicates that about half of the full sunlight of midday is sufficient for manifestation of photosynthetic ability of the plant just like in the case of isolated plant. However, as the growth proceeds the light requirement for full photosynthesis increases progressively, and after the young panicle formation stage the light-saturation points disappear even under full sunlight of midday. By these stages, as shown in Fig. 6, total leaf area increases so much that sufficient light can not penetrate into the

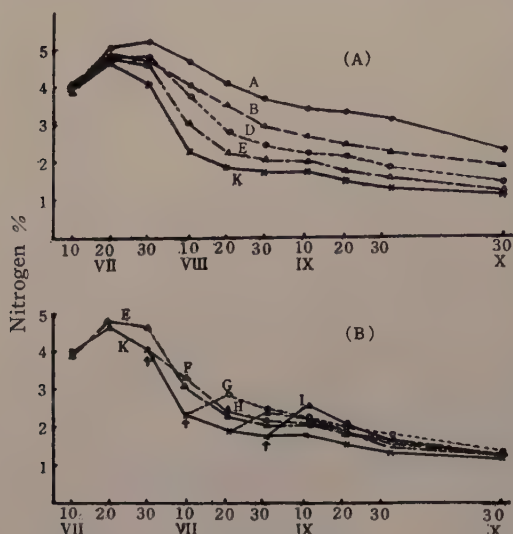


Fig. 8. Changes in nitrogen percentage in leaf-blade.

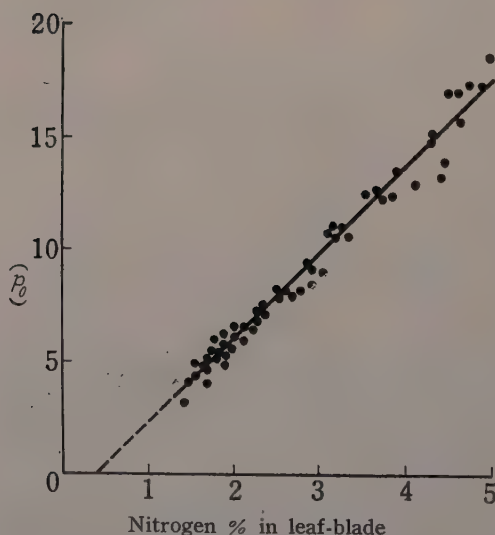


Fig. 9. Relationship between  $p_0$  and mean nitrogen content in leaf-blade.

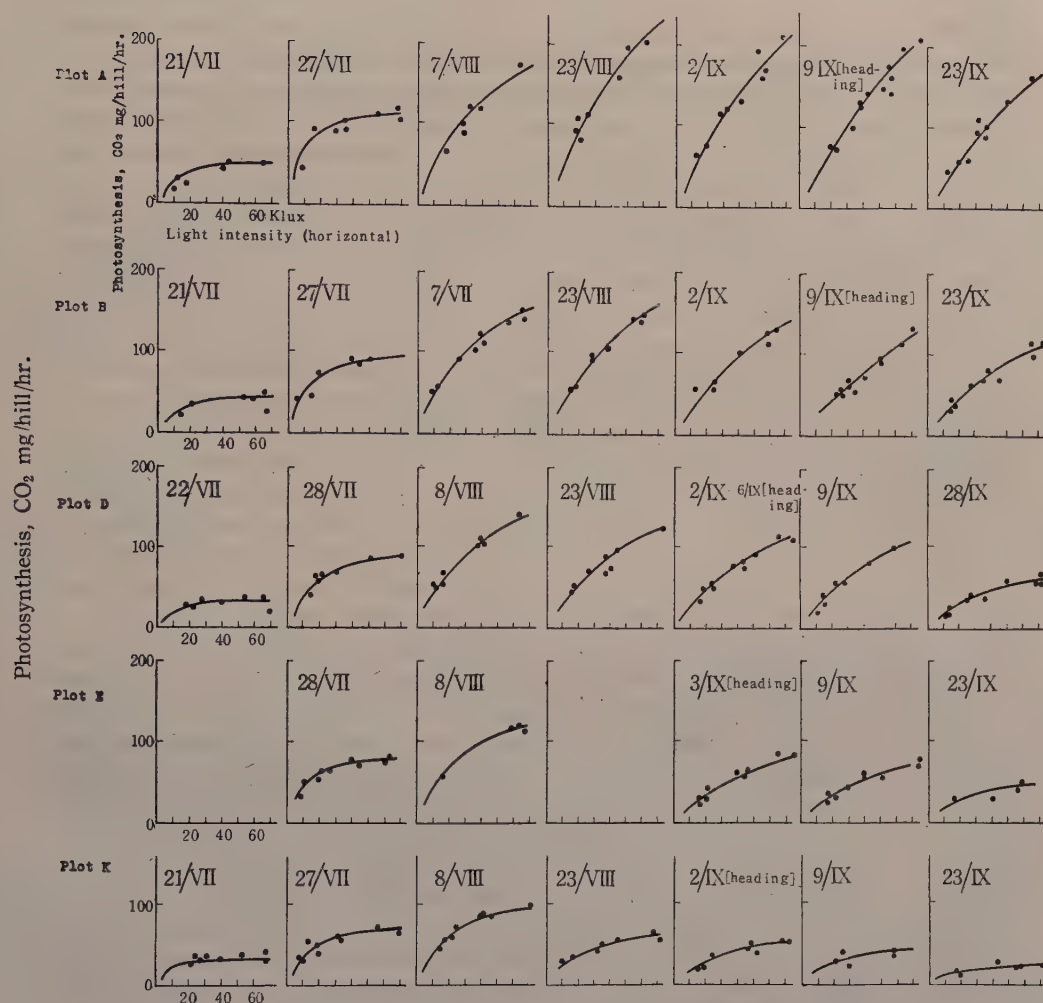


Fig. 10. Relationship between light intensity and photosynthesis of the plant growing in the field as affected by growth stages.

community. As the plant grows further, the light curves lose their curvature gradually, and tend to approach straight lines after the stage of booting. These trends are stronger in the plots fertilized with a larger amount of nitrogen. In these stages leaf area increases maximum and the penetration of light into the community becomes very slight. As shown in Fig. 13, the amount of light penetrating the surface of the community near the ground becomes least at this stage. On the other hand, in the lightly fertilized plots, compared with the heavily fertilized plots, the light curves are slightly curved throughout all growing stages. Such phenomena correspond to the fact that leaf area decreases with decrease of nitrogen fertilizer, and the projected light can easily penetrate into the community. In further advanced stage, at the end of ripening, the light



curves become slightly curved again. In this stage total leaf area decreases considerably, and the light intensity at the ground level increases a little again.

Now, it is clear that the relationships between light intensity and photosynthetic rate as affected by the growth stages and the amounts of supplied nitrogen fertilizer are always concerned with the degree of growth of the community. Concerning the cause of these observations, the author and his colleague [6] have considered as follows: in the case of isolated individual, as the plant can receive the light projected from all directions, which is so sufficient that saturation can be seen throughout all growing period. On the contrary, under the community conditions, especially after the middle stage of growth, leaf blades and stems are piled up together and the mutual shading takes place, as a result, the projected light can not easily penetrate into the community, accordingly, even under the full sunlight of midday, though the upper layer of the community receives sufficient light for full photosynthetic activity, a considerable part of inner portions is in shortage of light. Thus as a whole, the community conditions are inferior in the mean amount of light, received by individuals, to the isolated conditions.

Thus, throughout the period of growth after the elongation of internodes of stem the community of rice plants is in constant shortage of light even under the full sunlight of midday. During the physiologically important stages of rice plant, such as flowering and ripening stages, the photosynthesis increases with the increase of light intensity up to the full sunlight.

(4) Changes in light receiving coefficient ( $\rho$ ) with the growth.

From the light curves shown in Fig. 10, the changes of field photosynthetic activity under given light intensity can be obtained. Fig. 11 shows them under a certain strong light intensity condition (70 Klux of horizontal incidence). By comparing the relations shown in Fig. 5 with that of Fig. 11, it is recognized that as a whole under community conditions there are considerable reductions in the photosynthetic activity in comparison

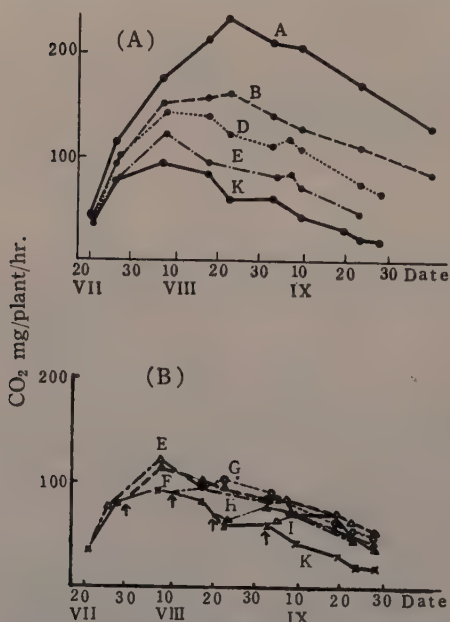


Fig. 11. Changes in field photosynthetic activity of the plant under community conditions with advancement of growth.

with isolated conditions. And the difference between  $P$  and  $P_0$  increases more and more with advancement of growth, and its maximum at about the heading stage. Comparing them at the same stage, it is clear that the more the amount of nitrogen supply (namely, the greater the total leaf area), the greater is the difference between  $P$  and  $P_0$ .

The reduction of  $P$  to  $P_0$  is due to the mutual shading caused by the

development of the leaves.

Thus, the ratio of  $P$  to  $P_0$  means the efficiency in the photosynthetic activity under community conditions, which is determined mainly by the way of receiving light. Hence, the author intends to call this ratio the "light receiving coefficient." And it can be expressed as follows:

$$\rho = \frac{P}{P_0} \quad (1)$$

where  $\rho$  denotes "light receiving coefficient." As seen from its nature, this coefficient must be in the range:

$$1 \geq \rho > 0 \quad (2)$$

Here,  $\rho$  is similar in meaning with that of  $f$  demonstrated by Murata et al [4]. However the procedures of obtaining  $\rho$  and  $f$  are quite different.

The changes of  $\rho$  at successive stages of growth in each plot are shown in Fig. 12. Comparing the curves of this figure with those of Fig. 6, it is evident that the changes of  $\rho$  with growth are in a reverse relation to the changes of  $S$ . In early stage of development,  $S$  is low and  $\rho$  is high. The increase of  $S$  with advancement of growth is accompanied by the decrease of  $\rho$ . After then, with decrease of total leaf area the value of  $\rho$  rises up slightly again.

By the above mentioned way, the author has come to the conclusions that the field photosynthetic activity ( $P$ ) of the rice plant forming a community under a certain light intensity condition can be approximately expressed by the multiplication of the following three factors: total leaf area ( $S$ ), photosynthetic

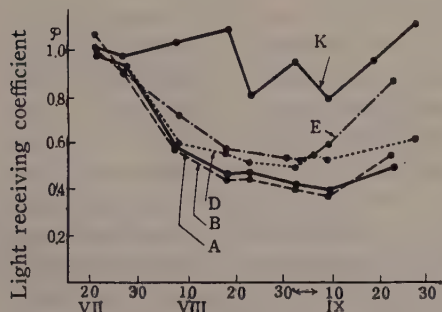


Fig. 12. Changes in light receiving coefficient (at 70 Klux) with advancement of growth.

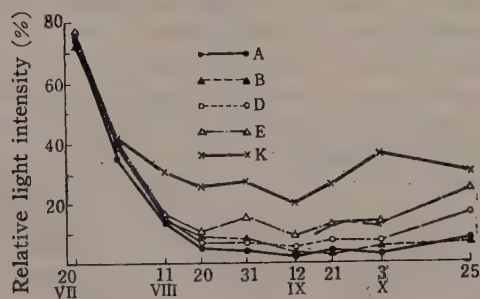


Fig. 13. Relative light intensity at the ground surface of the community.

ability per unit leaf area ( $p_0$ ), and light receiving coefficient ( $\rho$ ). And the relation can be expressed by the following equation:

$$P = S \cdot p_0 \cdot \rho \quad (3)$$

Fig. 13 shows the changes of relative light intensities measured at the ground level of each community at successive stages of growth, which indicated the interfering degree of the leaf layer for penetration of the projected light into the community. The relations shown in this figure resemble the changes of  $\rho$  with advancement of growth (Fig. 12). From this fact, it is clear that the changes of  $\rho$  are brought by the luxurious growth of the leaves.

As shown in Fig. 11, the changes in  $P$  with advancement of growth may be divided into the following three types:

- i. The type (a), which is highly active from full-tiller through heading stage, as seen in plot A supplied with heavy nitrogen.
- ii. The type (b), which has its maximum at full-tiller stage and then decreases, but revives again at about heading stage, as seen in plot D supplied with moderate amount of nitrogen and in plots *E* and *K* supplied with lower amount.
- iii. The transforming type (c) is between above two types, which keeps high activity from full-tiller through booting stage and then decreases gradually, as seen in plot *B*.

This changes can be explained by means of the above mentioned three factors as follows: in type (a), during early growing period  $P$  increases progressively and keeps its pace with that of  $P_0$ , because during this period total leaf area develops vigorously and the mutual shading of the leaves does not take place yet. In other words, the increase of  $P$  during this period is due mainly to the increase of  $S$ . After then  $P$  increases further. However, its rate of increment falls down gradually from that of  $P_0$ . As it is brought about by the increase of mutual shading owing to the further expansion of the leaves. The comparatively higher activity maintained by  $P$  from booting through heading stage may be explained as follows: the increase of  $S$  proceeding until heading stage prevents the declination of  $P_0$  due to the decrease of  $p_0$ . So  $P_0$  keeps its high capacity from booting through heading. On the other hand, the reducing degree of  $p$  declines steadily with further increase of  $S$ , hence, during certain range of comparatively larger  $S$ ,  $P$  is approximately proportional to  $P_0$ . As a result,  $P$  keeps its high activity during these stages. Thereafter, during ripening stage  $P$  decreases with the decrease of  $P_0$  owing to the decrease of both  $S$  and  $p_0$ . In type (c), changes of  $P$  in early stage and its maximum can be explained similarly like the case of type (a), and its decreasing trend after booting is brought about by the decrease of both  $S$  and  $p_0$ .

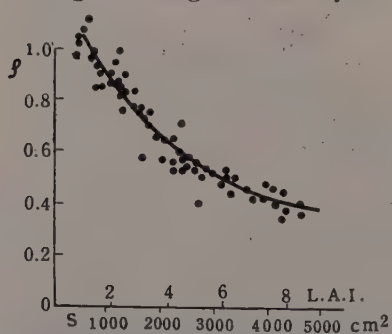


Fig. 14. Relationship between light receiving coefficient and total leaf-area.

In type (b),  $P$  is maximum at the full-tiller stage and then it decreases progressively. This decrease is caused by the fact that the negative effect of decrease in  $p_0$  upon  $P$  supersedes the positive effect of increase in  $S$ , and further that  $p$  decreases abruptly with increase of  $S$ . The gradual declination of the rate of decrease in  $P$  from booting through heading stage is caused by both the facts that the decreasing rate of  $P_0$  somewhat declines and that the reducing degree of  $p$  declines gradually accompanied by further increase of  $S$ . The changes in plot *K* supplied with lesser nitrogen resemble roughly with those of plot *D* and *E*. However, owing to comparatively small  $S$ , no mutual shading takes place and hence both the curves of  $P$  and  $P_0$  coincide approximately with each



other.

Considering mutual shading, it can easily be understood that the increments of leaves and stems are associated with the depression of light receiving coefficient. Thus, the relationship between  $S$  and  $\rho$  has been investigated. These relations are illustrated in Fig. 14. As shown in the figure, until leaf area index (LAI) attains ca. 2.0,  $\rho$  keeps its value near 1.0. As LAI increases beyond this point,  $\rho$  falls down abruptly. After then, increase of LAI is accompanied by decrease of  $\rho$ . However, the rate of decrease of  $\rho$  declines with the further increase of LAI. In other words, when total leaf area of individual plant is smaller than ca. 1200 cm<sup>2</sup>, mutual shading does not take place. Once it exceeds this limit, mutual shading takes place abruptly and increases progressively with further increase of total leaf area. But the rate of increase of mutual shading declines gradually with the further increase of leaf area. The author made clear that the relationship between total leaf area and light receiving coefficient can be expressed by the following equation:

$$\rho = 1.1068 \cdot 10^{-286} \cdot S + 352 \cdot 10^{-10} S^2 \quad (4)$$

where  $\rho$  is the light receiving coefficient,  $S$  is the total leaf area.

Owing to the reflection and absorption by leaves, light projected on the surface of the community reduces in intensity as it penetrates into the plant community. From the results of observation and the theoretical analysis of the relationship between leaf area index and the relative light intensities in various communities, Monsi and Saeki [3] made clear that the light intensity in a certain stratum in a community can be expressed by the following formula:

$$I = I_0 e^{-kF} \quad (5)$$

where  $I_0$  is the light intensity at the top of the community,  $F$  is the leaf area index from the top to the stratum in question,  $k$  is the extinction coefficient.

In this experiment, using an artificial community of rice plants, it was clearly demonstrated that the linear relationship existed between  $\log \frac{I}{I_0}$  and leaf area index (Fig. 15). The result indicates that the formula demonstrated by Monsi and Saeki [3] is applicable to the artificial community of rice plants.

The author intends to analyze the relationship between  $S$  and  $\rho$  by means of application of the above stated law.

At first, let us suppose

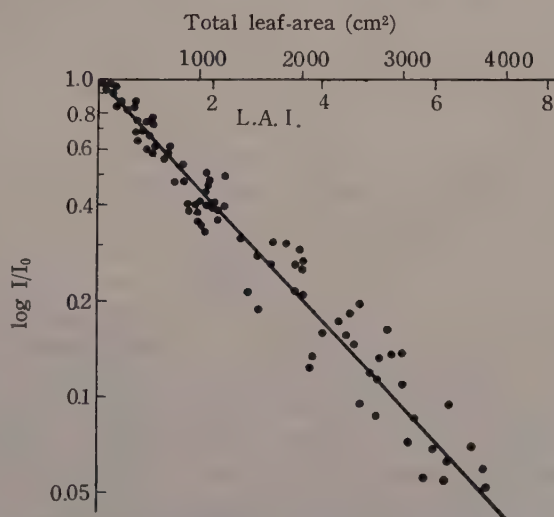


Fig. 15. Relationship between the amount of leaf-area and the relative light intensity in the community.



that  $\rho$  is determined only by the light conditions. Assuming it to be true, and considering that  $\rho$  is obtained from the ratio of  $P$  to  $P_0$ , it can be understood that the ratio of total magnitude of light received under community conditions to that received under isolated conditions indicates, so to speak, "light receiving state" ( $\sigma$ ). Here,  $\sigma$  is corresponding to  $\rho$ . If  $\rho$  is determined only by the light conditions, as postulated before, it may be expected that  $\sigma$  will coincide with  $\rho$ .

The light receiving state ( $\sigma$ ) may be expressed as follows:

$$\sigma = \frac{\text{total magnitude of light received under community conditions}}{\text{total magnitude of light received under isolated conditions}} \quad (6)$$

here, the numerator of equation (6), namely total magnitude of light received under community conditions ( $\Sigma P_i$ ) can be expressed as follows:

$$\Sigma P_i = \int_0^s I \cdot ds \quad (7)$$

where  $I$  denotes the light intensity in a certain stratum in the community,  $S$  is the total leaf area from the top to the stratum in question. From the formula demonstrated by Monsi and Saeki, it can be rewritten as follows:

$$\begin{aligned} \Sigma P_i &= \int_0^s I_0 e^{-ks} \cdot ds \\ &= I_0 \int_0^s e^{-ks} \cdot ds \end{aligned} \quad (8)$$

integrating this

$$= I_0 \left[ -\frac{e^{-ks}}{k} \right]_0^s \quad (9)$$

On the other hand, the denominator of equation (6), namely total magnitude of light received under isolated conditions ( $\Sigma P_{0i}$ ) can be expressed as follows:

$$\Sigma P_{0i} = I_0 \cdot S \quad (10)$$

From (9) and (10), the ratio of  $\Sigma P_i$  to  $\Sigma P_{0i}$  can be expressed as follows:

$$\begin{aligned} \frac{\Sigma P_i}{\Sigma P_{0i}} &= \frac{I_0 \left[ -\frac{e^{-ks}}{k} \right]_0^s}{I_0 S} \\ &= \frac{1}{kS} \left[ -e^{-ks} \right]_0^s \\ &= \frac{1}{kS} [-e^{-ks} + e^0]. \end{aligned}$$

here,  $e^0 = 1$ , then

$$\frac{\Sigma P_i}{\Sigma P_{0i}} = \frac{1}{kS} [1 - e^{-ks}] \quad (11)$$

Using the leaf area index and the relative light intensity shown in Fig. 14,

the constant  $k$  can be calculated with equation (5).

Using the equation (11) shown above and the value of constant  $k$ , the author calculated the ratio of  $\Sigma P_i$  to  $\Sigma P_{oi}$  corresponding to each leaf area, and showed the relationship between the ratio and total leaf area in Fig. 16. As shown in the figure, for example, when the leaf area index is ca. 6.6 (Namely, in this case it corresponds with individual total leaf area of 3500 cm<sup>2</sup>), the total magnitude of light received under community conditions attains only one third of under isolated conditions. Accordingly, this finding indicates the following fact: if  $\rho$  is determined only by the light conditions, as postulated before, field photosynthetic activity attains only one third of that under isolated conditions.

On the other hand, light receiving coefficient obtained from the actually measured value of  $P$  and  $P_0$  are shown with the broken line in Fig. 16. As shown in the figure, the curve resembles closely in its shape to that of light receiving state, however,  $\rho$  is always larger than  $\sigma$  throughout all the ranges. The result indicates that  $\sigma$  does not represent directly  $\rho$ . Accordingly, it can be said that the light receiving coefficient is not determined only by the light conditions. However, resemblance and parallelism in the shape of these two curves indicate that  $\rho$  has a close connection with  $\sigma$ . But in spite of their close connection, why there is discordance each other? The reason must be determined.

If one divides the factors concerning field photosynthetic activity into two main components, the internal and external factors, the light receiving state may rather belong to the latter. And the light receiving coefficient is no more than the expression that it is the resultant of interaction between the two. Considering such a fact, the above observed discordance must be explained by the internal factors.

Thus, the author intends to investigate the internal factors. As the factors being concerned mainly with photosynthesis, the following two are enumerated. The first is the relationship between photosynthetic ability of leaf blade and light intensity. The former is not always proportional to the latter throughout all ranges, but the light saturating phenomenon occurs at 30–40 Klux of light intensity. The second is the nitrogen concentration in leaf blade in respect of the height in the community.

Now, let us assume that the relationship between photosynthetic rate of leaf blade and light intensity can be expressed by Fig. 17, the assimilation layer receiving light over 30 Klux can manifest the full capacity of photosynthesis,

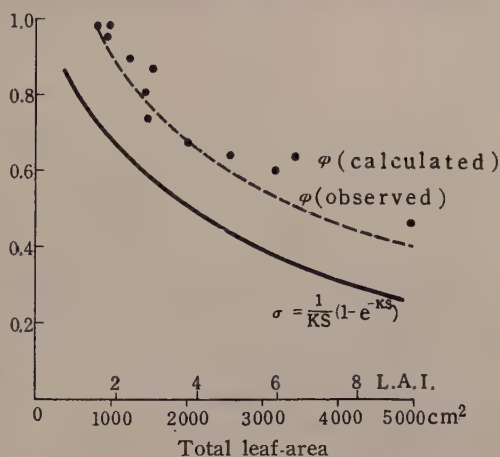


Fig. 16. Relationship between total leaf-area and  $\rho$ ,  $\sigma$ .

but the layer receiving light below 30 Klux performs the photosynthesis in proportion to the light intensity.

The contents of nitrogenous compounds in the photosynthetic layer differed comparatively with the height of the community (Fig. 18). In heavily fertilized

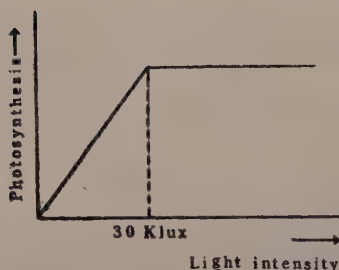


Fig. 17. Relationship between light intensity and photosynthetic ability of the leaf.

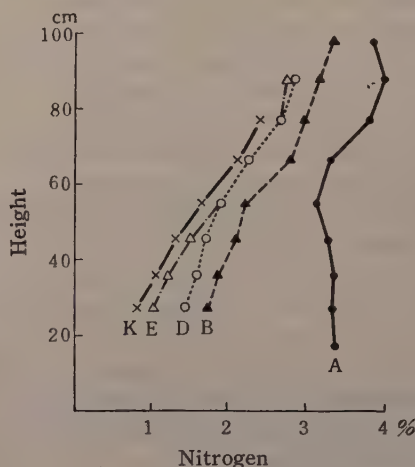


Fig. 18. Changes in nitrogen % in leaf-blade with the height of the community.

plot, the difference of the nitrogen contents between upper and lower layer is not so distinct. But in the case of moderately or lightly fertilized plot, the contents of upper layer are clearly higher than those of lower layer. Since the photosynthetic ability of leaf blade, as shown in Fig. 9, has a high positive correlation with the nitrogen concentration of the leaf blade, it may be ascertained that the upper layer has a higher photosynthetic ability than the lower one. Hence, it can be expected that the leaves of the lower layer, where the depression of light intensity is especially heavy, have comparatively light effect upon the decrease of photosynthetic rate.

Now, let us assume that the photosynthetic ability of leaf blade under saturated light intensity is determined by the relation shown in Fig. 9.

The photosynthetic rate of each layer can be calculated, and the integration of them is the total photosynthetic rate of a whole plant. Thus, this value is the theoretical one corresponding to the measured value of the field photosynthetic activity ( $P$ ) under community conditions. On the other hand, the value calculated in the similar way of the total photosynthetic rate when light was more than the saturation point is the theoretical one corresponding to that of photosynthetic capacity under isolated conditions. The ratio of the theoretical  $P$  to theoretical  $P_0$  is the value of theoretical light receiving coefficient corresponding to the measured value of  $\rho$ . As for example, the calculated values of  $\rho$  in the stage of heading are shown with dots in Fig. 16. As shown in the figure, calculated values are nearly equal to the measured ones. The relationship between theoretical values and the measured ones is shown in Fig. 19. It is

clear that a high positive correlation exists between them. In other words, these facts indicate that the "light receiving coefficient" can be induced theoretically from the following three factors: the light receiving state, the light-photosynthesis curve of leaf blade and the nitrogen distribution in each layer of the community.

In this way, the author has come to the following conclusions.

The non-linear decrease of the reduction of  $\rho$  with the increase of total leaf area comes from the fact that the total amount of light received by the plant does not decrease so much as the increase of total leaf area. The fact that the values of  $\rho$  being always higher than the ones of  $\sigma$  is supported both by the fact of light saturation phenomenon relating light intensity and photosynthetic ability of leaf blade, and of the difference of nitrogen distribution in each layer of the community.

That the rate of reduction of  $\rho$  declines steadily with the further increase of total leaf area, may be thought to be an adaptation. If this is the case, wherein lies the adaptation? The author considers as follows: it is based conclusively on the fact that the penetration of light into the community, as stated before, is subjected to the law represented by  $I = I_0 e^{-kF}$ —which is merely physical one.

## II Dry matter production in the community of rice plants

Change in the dry weight of the rice plant in each plot grown under the artificial community conditions are illustrated in Fig. 20. Figure (a) is that of the basal-fertilized plot, (b) and (c) are that of the top-dressed plot. Since this experiment is performed, as stated before, under constant spacing (18,000 hills per Tan ( $\approx 10$  a)) throughout all plots, these growth curves represent consequently the relations per unit field area.

From this figure, it may be said that the growth of rice plants under community conditions is slow in the early growth stage, becomes vigorous in

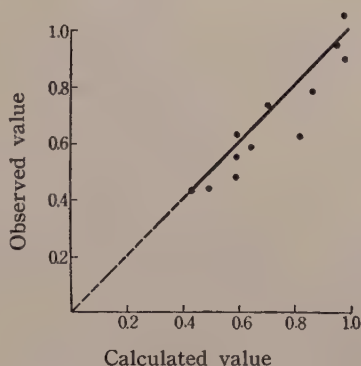


Fig. 19. Relationship between calculated and observed value of  $\phi$ .

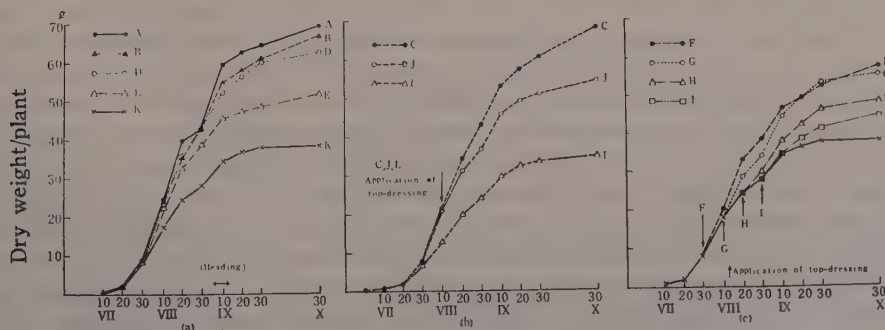


Fig. 20. Growth curves of the plant.



the middle, and then slows down again in the later stages, and accordingly, the growth curve takes approximately the shape of a sigmoid. However, its procedure is by no means so simple. All plots show a sudden decrease in the rate of dry matter production at the booting stage (from 20 Aug. to 30 Aug.). Rewriting

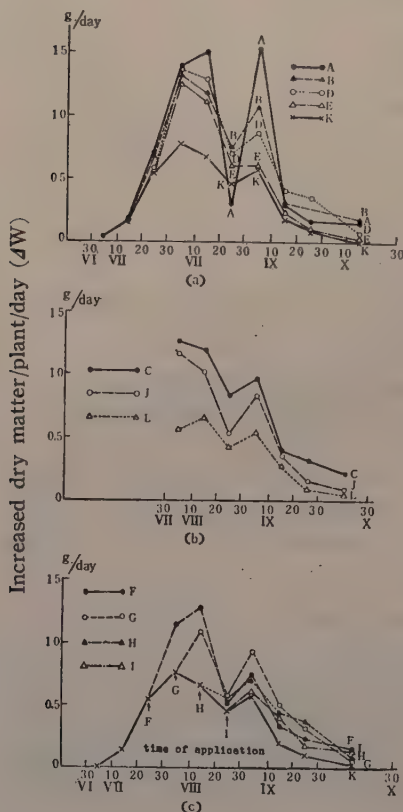


Fig. 21. Changes in increasing rate of dry weight with advancement of growth.

rate of increase of dry matter takes place simultaneously in all the plots during booting stage. Now the question arises, whether the factors determining dry matter production belong to internal factor or to the environmental conditions including radiation, temperature and others, and alternatively, whether it comes from the interaction between plant factors and environmental ones. If it be so, are there any differences in the way in which these factors interact each other during the advancement of growth stages? The author intends to put the analysis forward concerning the above mentioned problems.

As already clarified, the plant factors, which determine the field photosynthetic activity, are the total leaf area ( $S$ ), the photosynthetic ability per unit leaf area ( $p_0$ ) and the light receiving coefficient ( $\rho$ ). However, the actual photosynthetic

above figure by the rate of increase in dry matter per day, these trends are shown further clearly (Fig. 21). In the basal-fertilized plot, the difference between each plot is extremely small during early growth period, after then as the growth proceeds the rate of increase in dry matter becomes larger in proportion to the amount of basal nitrogenous fertilizing, and then at the booting stage the rate of increase of each plot decreases abruptly. The fact that the rate of increase of dry weight in the heavily fertilized plot (plot A) is smaller than that of the lightly fertilized one (plot K) can not be accepted easily. Thereafter in the heading stage it recovers again in each plot, and after then decreases considerably during ripening. The nitrogen top-dressing always brings about considerable increase of dry matter production. The earlier the time of application, the higher the efficiency of top-dressing. The abrupt decrease of the rate of increase in dry matter at the stage of booting and ripening can also be observed just like the case of basal fertilization.

As described above, the procedure of dry matter production is affected by the amount of basal-fertilization and the time of application of top-dressing. However, in any case, the abrupt decrease of the

rate performed by the plant under field conditions is affected by the climatic conditions (especially by the radiation). Thus, the relationship between the above stated factors and the production of dry matter will be examined in the following contexts.

(1) Relationship between leaf area ( $S$ ) and dry matter production.

Fig. 22 shows the relationships throughout all growth stages, on abscissa, mean leaf area ( $S$ ) for 10 days in each stages, and on ordinate, mean dry matter production per day ( $\Delta W$ ) for the same period are plotted. Where,  $S_1$ , denotes

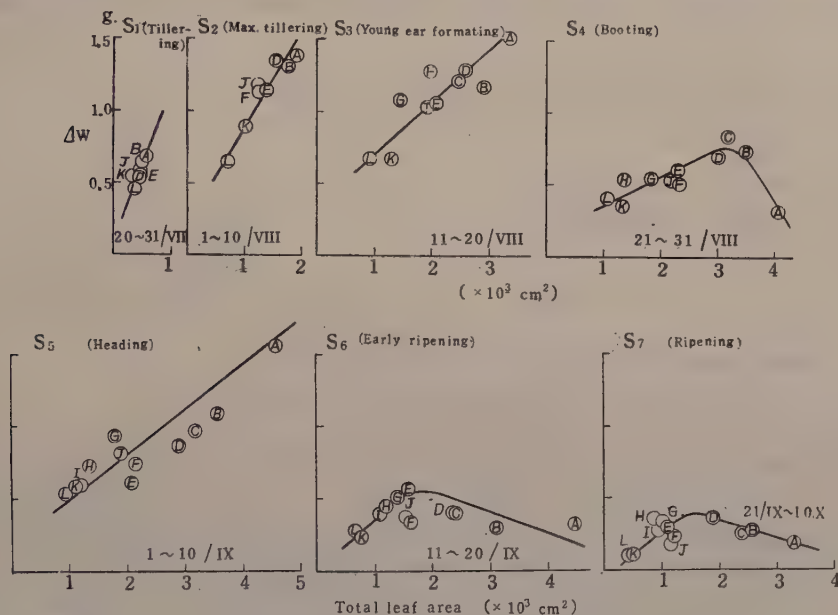


Fig. 22. Relationship between leaf-area ( $S$ ) and dry matter production.

tillering stage;  $S_2$ , maximum tillering;  $S_3$  young panicle forming;  $S_4$ , booting;  $S_5$ , ripening. The marks  $ABC.....L$  in the figure denote the plots supplied with various amount of nitrogen fertilizer (cf. Table 1). As shown in the figure, in tillering stage, namely in early stage, the linear relationship between  $S$  and  $\Delta W$  is of sharp gradient, afterwards its gradient becomes gentle gradually with advancement of growth stages. In other words, the regression coefficient of  $S$  to  $\Delta W$  is larger in early stage, but it becomes smaller with advancement of growth stages. Such changes mean that in early growing stage, the effect of the change in leaf area upon dry matter production is greater than in later stage, and this efficiency decreases gradually with growth.

On the other hand, in  $S_4$ — $S_6$ — $S_7$ —stages the linear relationships between  $S$  and  $\Delta W$  cannot be seen, like the other stages. Up to a certain limit, the more the leaf area increases, the more the dry matter is produced. However, when leaf area increases beyond this limit, produced dry matter decreases progressively. Consequently the linear relationships bend down in half-way. Furthermore, the turning point, at which dry matter productivity either increases or decreases,

lies at about 3,300 cm<sup>2</sup> of leaf area (LAI=ca. 6.0) in  $S_4$  stage. In  $S_6$ — $S_7$ —stages, the turning points lie at about 1,600 cm<sup>2</sup> (LAI=ca. 3.0). Namely it is removed in the direction in which leaf area decreases with advancement of growth stages.

(2) Relationship between photosynthetic ability on leaf area basis ( $p_0$ ) and dry matter productivity.

Fig. 23 shows the relationships between the mean value of  $p_0$  for short interval (10 days) at successive growth stages and the mean increase in dry weight per day during similar interval. Where, marks  $p_{01}$ ,  $p_{02}$ ..... $p_{07}$  correspond to the signs in leaf area, and represent the growth stages respectively. According

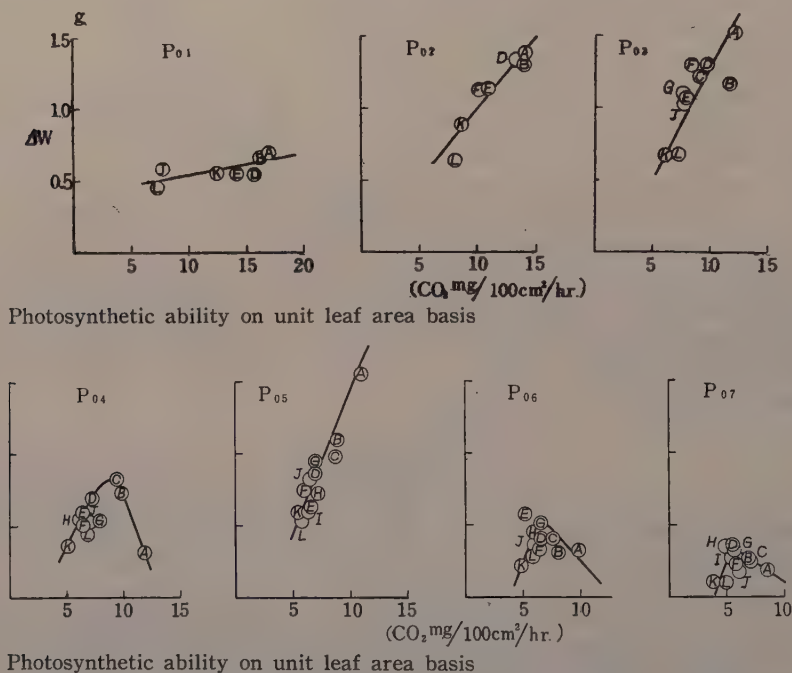


Fig. 23. Relationship between photosynthetic ability per unit leaf area and dry matter production.

to the figure, in early growth stage the regression coefficient of  $p_0$  to  $\Delta W$  is smaller, but becomes larger progressively with advancement of growth stages. Such tendencies with growth stages mean that in early growth stage the change in  $p_0$  has a lesser effect upon dry matter production than in later stages, and this efficiency increases progressively with advancement of growth stages.

On the other hand, in some stages ( $p_{04}$ ,  $p_{06}$ ,  $p_{07}$ ) the linear relationships between  $p_0$  and  $\Delta W$  cannot be seen. When  $p_0$  increases beyond a certain limit, the rate of dry matter production rather decreases progressively. And the turning points which lie at about 9, 7, 6  $\text{CO}_2 \text{ mg}/100 \text{ cm}^2/\text{hr.}$  respectively, are removed with advancement of growth stages in the direction in which  $p_0$  decreases.

(3) Relationship between field photosynthetic activity ( $P$ ) and dry matter productivity.

Changes of relationship between  $P$  and  $\Delta W$  at different times in the growth period have been investigated. Fig. 24 shows this relation. A certain trend of

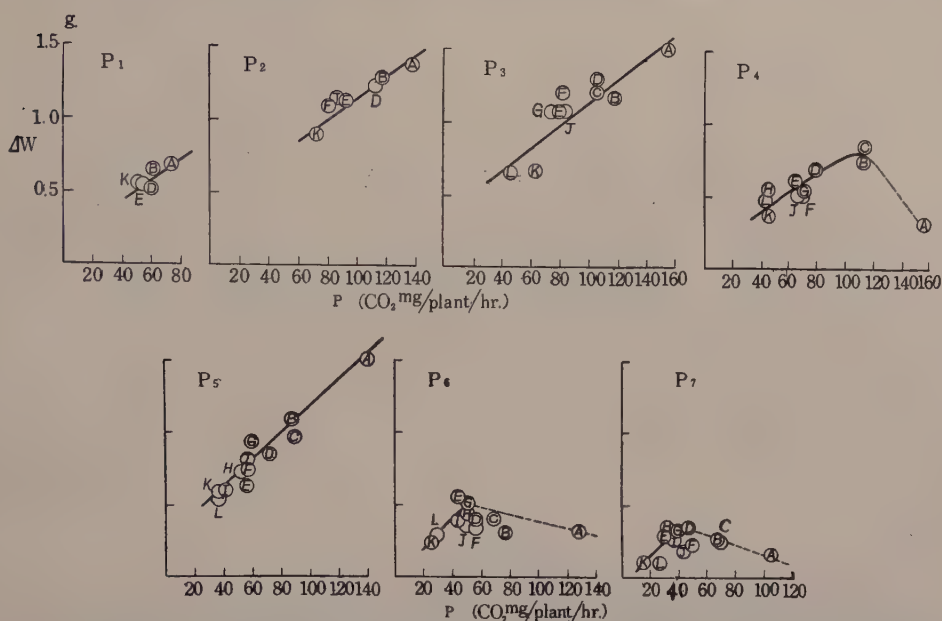


Fig. 24. Relationship between field photosynthetic activity and dry matter production.

changes in relationship with advancement of growth stages cannot be seen, like the cases of  $S$  and  $p_0$ . As already stated,  $S$  and  $p_0$  are involved in  $P$  in the form of multiplication. As a result, it may be accounted for the cancelation of the effects of  $S$  and  $p_0$  by each other.

On the other hand, in  $P_4$ – $P_6$ – $P_7$ —stages the linear relationship can not be seen, like the cases of  $S$  and  $p_0$ . Up to a certain limit, the more the  $P$  increases, the more the  $\Delta W$  is produced, but when  $P$  increases beyond the limit,  $\Delta W$  decreases gradually with advancement of growth stages, like the cases of  $S$  and  $p_0$ .

Here, a question arises as to why these phenomena take place. Such facts were seen in every case, such as  $S$ ,  $p_0$  and  $P$ , but its reasons could not be clarified in all cases.

The analysis attempted in the paragraphs above are all concerned with only the factors belonging to the plant, and the ones belonging to the environment are not considered yet. Accordingly, if one attempts to make the reason clear, the phenomena must be analyzed in consideration of the environmental factors.

(4) Relationship between field photosynthetic activity ( $P$ ) and dry matter productivity with regards to environmental factors.

The rate of photosynthesis performed under natural conditions are determined by the two main factors, the field photosynthetic activity and the light conditions; the former belongs to the plant, and the latter, to the environment.



Fig. 25 illustrates the climatic conditions during experimental period. As shown in the figure, total radiation fluctuated irregularly day by day, however, mean radiation during 10 days in 1, 2, 3, 5, stages was nearly constant, about

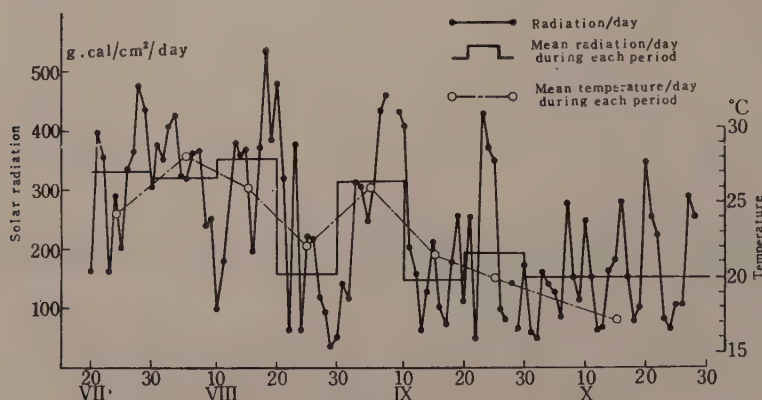


Fig. 25. Climatic conditions during experimental period.

310-350 cal/cm<sup>2</sup>/day in each stages. On the other hand, in 4, 6, 7 stages it fluctuated within the range of 150-190 cal/cm<sup>2</sup>/day owing to duration of cloudy or rainydays.

Comparing the climatic conditions with the procedure of dry matter production, it can be found that the periods which received lesser amount of mean radiation corresponded with the periods which produced lesser amount of dry matter. In other words, it seems that climatic condition is one of the joint

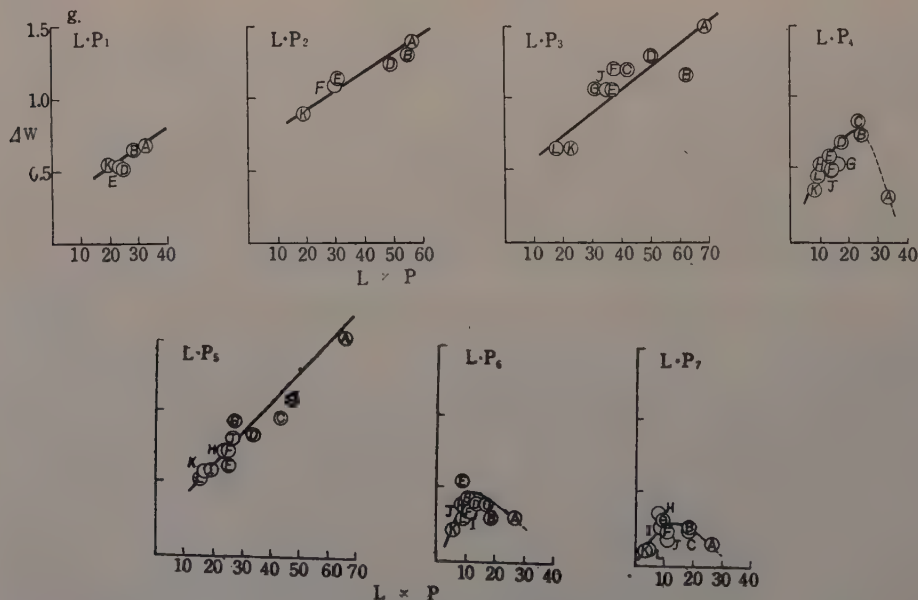


Fig. 26. Relationship between ( $L^* \times P$ ) and dry matter production.

\* Mean radiation, per day during 10 days.

factors in decreasing dry matter production.

The relationship between  $P$  and  $\Delta W$  with regards to the environmental factors has been examined.

Fig. 26 shows these relationships. From the figure it can be found that the same phenomenon, as seen before, occurs in the 4, 6, 7 stages. The relationships are not always linear throughout all stages. From this fact, it can not be assumed that the decrease of dry matter production comes only from the decrease in photosynthetic rate due to the unfavourable climatic conditions.

There are no factors which can determine the field photosynthetic rate other than the above examined ones. Accordingly, the explanation of such a relationship must require some factors other than the photosynthesis.

(5) Relationship between respiration rate and dry matter productivity.

Here, as the factors concerning dry matter productivity other than the factors which determine the field photosynthetic rate, the respiratory ability and the temperature determining the rate of respiration are enumerated.

The amount of  $\text{CO}_2$  assimilated by the plant is corresponding to the gross

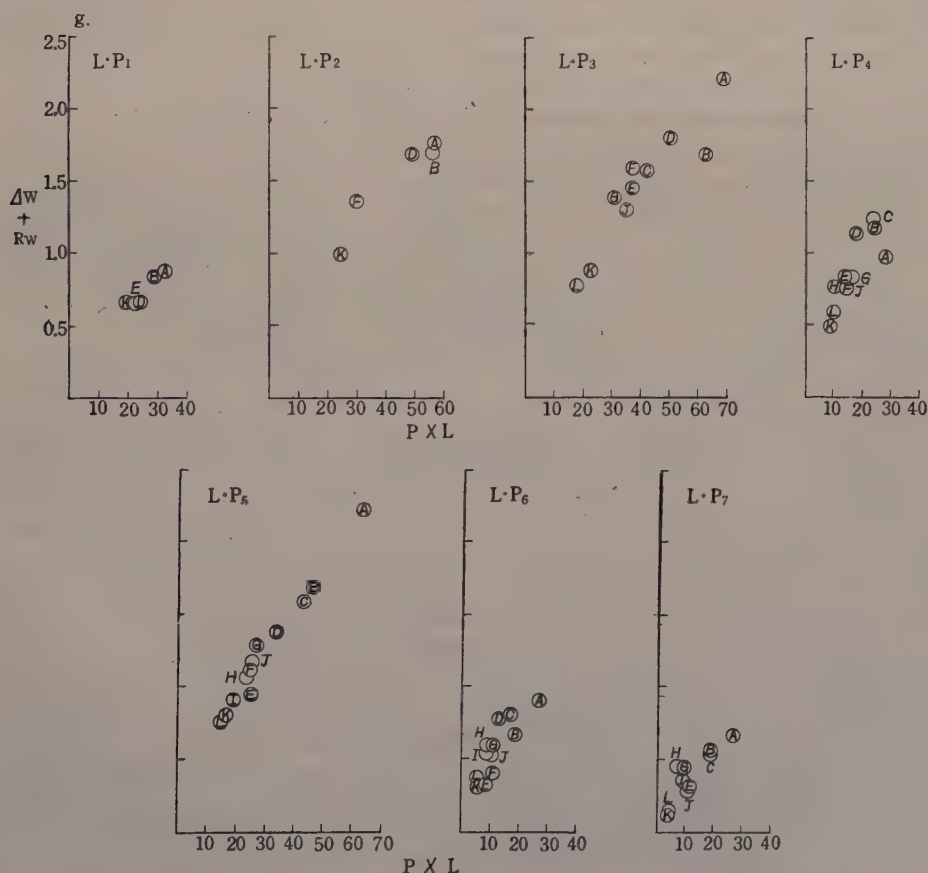


Fig. 27. Relationship between  $(L \times P)$  and  $(\Delta W + R_w)$ .

assimilation, and the produced dry matter is the resultant of photosynthetic gain and respiratory loss. It may therefore be understood that the factors determining dry matter production are not only the factors constituting field photosynthetic rate, but also the respiratory ability and temperature which decides the rate of respiration. Thus, the relationships between respiration rate and dry matter production have to be examined.

From the results illustrated in Figs. 5 and 2, it is evident that the  $P_0$  decreases abruptly after heading, however the respiratory ability are kept considerably at high level from the stage of booting to heading and ripening. Such a difference between the changes in photosynthetic capacity and respiratory ability with growth stage affects seriously the production of dry matter. During investigation of the relationship between respiration and dry matter productivity, it must be noticed that the respiration rate varies with the temperature.

Fig. 27 illustrates the relationship between field photosynthetic rate ( $P \times L$ ) and dry matter production associated with respiratory loss ( $\Delta W + R_w$ ). The mean respiration rate per day during each growth period is calculated from the respiratory ability by using the mean temperature during the same period and the temperature coefficient of respiration ( $Q_{10}=2.0$ ). These values are converted into the dry matter by means of the general formula of carbohydrate ( $C_6H_{10}O_5$ )<sub>n</sub>, and are multiplied by 24 hrs, to obtain the respiratory loss per day ( $R_w$ ). As shown in the figure, no bending off in half way of the regression line between

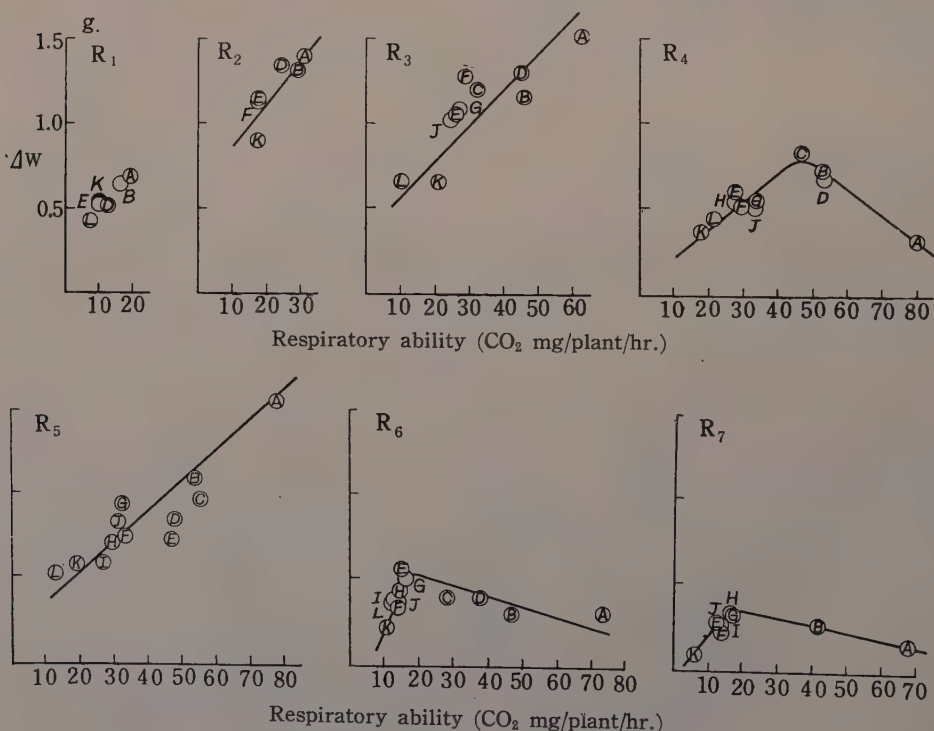


Fig. 28. Relationship between respiratory ability and dry matter production.

( $\Delta W + R_w$ ) and ( $P \times L$ ) at the 4, 6, 7 stages can be found. And the relationships throughout all growth stages can be shown by a line.

Then the author intends to examine the relationship between respiratory ability and dry matter production. From Fig. 28, it is evident that in the 4, 6, 7 stages the respiratory ability over a certain limit results in the decrease of dry matter production, and the turning point at which the dry matter production either increases or decreases moves with advancement of growth stages in the direction in which the rate of respiration decreases. The following hypothesis may be justified by these results. The reason which can not be explained in above paragraphs comes from fact that in these stages photosynthetic rate decreases due to unfavourable climatic conditions, and the respiratory loss appears on the way, therefore, the dry matter production is determined mainly by the respiration rate especially in the plot supplied heavily with nitrogenous fertilizer. In other words, the decreases of photosynthetic rate are caused by the unfavourable climatic conditions and by the comparatively high amount of respiratory loss.

From the results analyzed above, the author has come to the conclusions that when the climatic conditions are favourable, dry matter production is determined mainly by the photosynthetic activity. Accordingly under such conditions the higher the photosynthetic activity, the more the production of dry matter. On the contrary, under an unfavourable climatic conditions it is determined strongly by the respiration rate. In the community possessing higher photosynthetic activity, the leaf area, the degree of mutual shading, and the respiratory ability are greater. Therefore, once the radiation is reduced, the rate of photosynthesis falls down rapidly, but the rate of respiration does not decrease so much, as a result the produced dry matter decreases strongly.

Thus, under unfavourable climatic conditions, especially under weak radiation, the community possessing lower photosynthetic activity produced rather more dry matter than that of higher photosynthetic activity (Fig. 21).

The fact that under unfavourable conditions every factor concerning dry matter production turns in the direction in which they decrease with advancement of growth stage, may be explained as follows: at first, the larger the mutual shading the lesser the photosynthetic rate. In contrast, the respiration rate is not affected by unfavourable conditions so much as the former. The larger the mutual shading, the higher the respiration rate. Since the produced dry matter is the remainder of photosynthetic gain and respiratory loss, the maxima of dry matter production should be shifted in the direction of the larger remainder, or of lesser mutual shading, with worse climatic conditions. The ratio of  $P_0$  to  $R$  decreases gradually with advancement of growth stages, such as 5.05, 7.30, 5.53, 4.48, 4.30, 3.20, 3.86 respectively. As a result, under unfavourable climatic conditions the maxima of dry matter production moves in the direction in where the decrease of photosynthetic rate becomes lesser, namely where the mutual shading becomes lesser,  $P_0$ ,  $P$  and  $R$  are lesser.

(6) The general formulal of dry matter production.

Putting the relationships illustrated in Fig. 27 in a single figure, Fig. 29 can be obtained. According to the figure, the sum of dry matter produced and



consumed has generally a high positive correlation with the multiplication of radiation and field photosynthetic activity throughout all growth periods.

From the result, we can get the following equation:

$$\Delta W + R_w \propto L \cdot S \cdot p_0 \cdot \rho \quad (12)$$

thus, from (12), the general formula of dry matter production in the community of rice plants can be expressed as follows:

$$\Delta W = k \cdot L \cdot S \cdot p_0 \cdot \rho - R_w \quad (13)$$

( $k$  = a constant)

From the above result, it can be deduced experimentally that the dry matter production is the remainder resulted from the deduction of the consumed dry matter from the sum of produced dry matter, i.e. the multiplication of leaf area, photosynthetic ability per unit leaf area, light receiving coefficient, and total radiation.

In above paragraphs, the author has inferred that the depression caused by unfavourable climatic conditions on dry matter production was on account of the decrease in photosynthetic rate as well as of the comparatively large amount of consumption by respiration. This inference may be proved by the following way.

Substituting the measured values in each stage by the factors of formula (13), one can obtain concrete mean values of constant ( $k$ ). At that time it was assumed that the radiation in stages 4, 6 and 7, as well as in the other normal stages, was 350 cal/dm<sup>2</sup>/day and the mean temperature was 26°C in stage 4 and 24°C in stages 6 and 7 in mid-September.

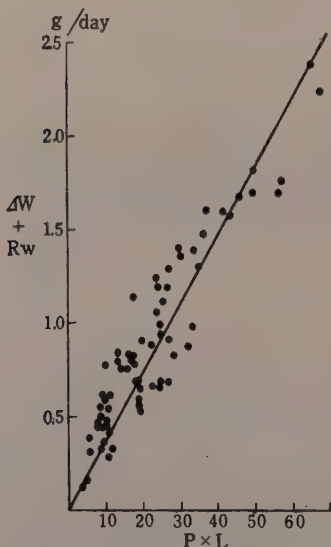


Fig. 29. Relationship between  $(\Delta W + R_w)$  and  $(P \times L)$ .

Fig. 30 shows the relationship between the factors constituting dry matter productivity and the produced dry matter that is calculated by means of the above stated procedure.

From the figure, it is evident that at any stage, if the climatic conditions are favorable, the produced dry matter increases in accordance with the increase of every factor constituting dry matter productivity. From these facts, it can be proved theoretically that the depressions of dry matter production shown in stages 4, 6, and 7 are caused fundamentally by unfavourable climatic conditions, i.e. deficiency of radiation, owing to decrease of photosynthetic rate and comparatively high loss due to respiration.

### III Conclusion — Suggestion for paddy field practice

In the foregoing paragraphs, it has been demonstrated that the factors constituting field photosynthetic ability namely  $S$ ,  $p_0$ ,  $\rho$ , and radiation have been

included in the form of multiplication in the general formula of dry matter production, and the rate of respiration, in the form of subtractor.

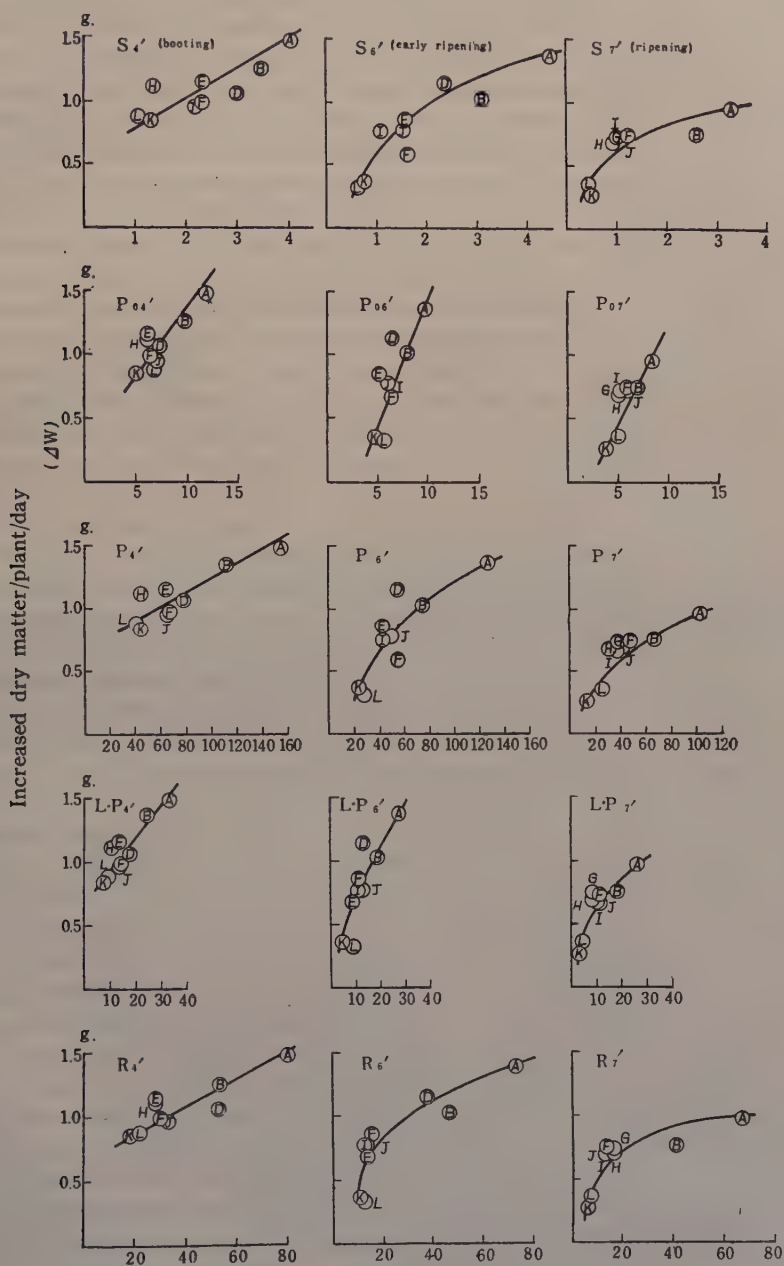


Fig. 30. Relationships between dry matter productivity and related factors under a favourable climatic-conditions.

However, if one try to set this analysis forward, one will face a few problems which must be taken into consideration. The first problem is whether the method of correlation analysis used by the author is applicable to such a case or not. Studying the relationships between each factor constituting dry matter production and the amount of produced dry matter, it may be ascertained that the leaf area has a high positive correlation not only with the respiratory ability, but also with the photosynthetic activity. It is needless to say, that the leaf area, photosynthesis and respiration are the outcome of many physiological processes which are combined together in the plant body, and go on simultaneously. Hence, it may be said that these factors are perfectly dependent variables. If it be so, application of the correlation analysis to such a case may not be regarded as the best method. In order to elucidate the influence of the special factor, it is necessary to conduct it by means of partial correlation analysis under separation from the influences of the other factors. Nevertheless, the author does not dare do so, only because of the fact that the analysis from concrete phenomena is to be started. The second problem is whether the relations between the growth stages are comparable or not. As seen in this experiment, if the environmental conditions in each stage are different from each other, it is not only difficult to compare the relationships between each other, in the strict sense of the word, but also becomes meaningless. If one attempts to compare each other, at least, the plants must be grown under considerably constant conditions throughout all growth stages. Only in such a case, the finding out the differences of characteristics in each growth stage might be expected. If one compares each other in the results obtained under randomly fluctuated conditions, not only the phenomena are complicated, but also analysing them is troublesome, consequently no law can be deduced from only the description of phenomena. For these reasons, it seems that the only way to reach a general law is that, the data observed under unfavourable climatic conditions of stages 4, 6, 7 in Figs. 22-28 are to be replaced by the values of Fig. 30 which were calculated with an assumption of normal climatic conditions, because with this procedure one can easily compare the relationships between respective growth stages and find out the differences of characteristics among the stages.

From the point of view stated above, the characteristics of each factor with advancement of growth stages have been investigated.

(1) Importance of leaf are and photosynthetic ability on a leaf area basis to rice cultivation. As shown in the relationship between  $S$  and  $\Delta W$ , the regression coefficient of  $S$  to  $\Delta W$  is larger in early growth stage, however it becomes gradually smaller with advancement of the stages. And in the ripening stage it becomes slightly curved. On the other hand, from the relationship between  $p_0$  and  $\Delta W$ , it can be seen that in the early stage the regression coefficient is smaller. However, the effect of  $p_0$  upon  $\Delta W$  becomes larger with advancement of growth. After middle stage, especially in later stages, it becomes very large. And the relationship between  $R$  and  $\Delta W$  remains linear till the heading stage, after then it becomes gradually curved.

These changes with growth stages indicate the following facts. In early stages the dry matter productivity in rice community is determined mainly by

total leaf area, but with advancement of growth stage this effect becomes gradually smaller. On the other hand, in early stages of growth the effect of photosynthetic ability on a leaf area basis is not so much upon dry matter productivity, and with advancement of growth stage it becomes larger gradually. After middle stages, especially in the later stages, dry matter production is determined mainly by the photosynthetic ability on a leaf area basis. The reason why the effect of leaf area on dry matter production decreases after middle stages, and the correlation line between them becomes curved, are explained as follows. As regards photosynthesis, increments of leaf area over a certain limit result in increase of mutual shading. Accordingly the field photosynthetic ability attains a plateau, and after then it can not increase anymore. On the other hand, with respect to respiration after the booting stage, the non-photosynthetic organ occupies a greater part of total weight and the photosynthetic ability falls down considerably. So the ratio of photosynthetic ability to respiratory ability decreases, and accordingly the consumption by respiration increases. As the respiration rate increases linearly in proportion to the increment of leaf area, dry matter production as a remainder does not increase linearly in proportion to increment of leaf area, so the relationship between  $S$  and  $\Delta W$  becomes rather curved.

Although the controlling effects of respiration upon dry matter production increase considerably in later part of growth periods when the mutual shading is increased, but the favourable climatic conditions are able to overcome these effects with high photosynthetic rate and enough to make it unobscure. However, under unfavourable climatic conditions, on account of abrupt decrease of photosynthetic rate accompanied by large respiratory loss, dry matter production decreases greatly in the community possessing larger leaf area over a certain limit. According to the results of the above-mentioned analysis, it can be understood that the decrease of dry matter production in the booting stage (cf. Fig. 21) and especially the higher dry matter production in the plots supplied with lesser nitrogen fertilizer than those supplied with more nitrogen fertilizer are rather due to the results brought about by the interaction of plants and climatic conditions, than merely by the nature of the plants themselves. In other words, it may be considered that the irregular fluctuations of produced dry matter are the results brought about by interaction of climatic conditions and the structure of plant community.

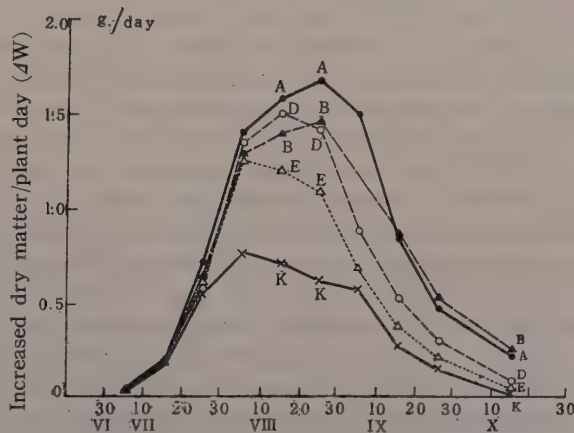


Fig. 31. Changes in increasing rate of dry weight with advancement of growth under favourable climatic conditions.



If it be so, the following problems will come on the way. When the changes of climatic conditions, which make dry matter production unstable, are avoided, how the plants display their productivity of dry matter attributed to themselves. These relationships have been investigated by means of "general formula." Supposing that total radiation throughout all growth period are  $350 \text{ cal/cm}^2/\text{day}$  (which is set on the basis of mean value of July and August in Japan), the changes of dry matter productivity according to growth stages are shown in Fig. 31. From the figure, it can be seen that dry matter productivity is relatively small in early stage, vigorous in middle, after then falls down again in later stage, and has a shape like a monomodal curve. In general, the more amount of nitrogen supplied, the greater the dry matter produced. And the stage at which production of dry matter attains its maximum is delayed with increasing fertilization. From these facts, it can be ascertained that the plant growth under constant climatic conditions may be represented by a sigmoid curve.

How the conclusions obtained from above stated discussion can be connected with the practice of rice cultivation?

Firstly, since the effect of leaf area upon dry matter production is greater especially in early stage, it is necessary to expand leaf area in early stage. Therefore, it is desirable to adopt the cultivation method promoting extension of leaf area, for example, supplying immediately effective fertilizer, and rising temperature of irrigation water. Schwarze [5] has pointed out this fact, and considered as an ideal type of high yield variety "to get rid of the stage possessing poor leaf area as soon as possible, and to develop rapidly extensive leaf area, and keep this area as long as possible."

Secondly, it is desirable to devise a countermeasure in order that photosynthetic ability on a leaf area basis may not fall down in later growth stage, for example, supplying gradually effective fertilizer, and preventing root damage; besides, promoting its ability positively, for example, top-dressing in later stage.

(2) Optimum leaf area for production of dry matter.

At the middle and later stages where the effect of leaf area upon the production of dry matter decreases, leaf area reaches its maximum, and mutual shading is heaviest, furthermore non-productive organs increase and consequently respiratory ability reaches its maximum. Larger leaf area means, in general, higher potential of productivity of the dry matter. Under a favourable climatic conditions, the community with larger area leaf can produce larger amount of dry matter. The effectiveness of leaf area in the production of dry matter, however, depends on the climatic conditions. The larger leaf area is not always beneficial to the production of dry matter. Under unfavourable climatic conditions, smaller leaf area is rather beneficial than the larger one. Considering this fact, it might be expected that the production of dry matter might vary with both the expansion of leaf area, and the climatic conditions.

For the purpose of quantitative analysis of the relationships between leaf area and climatic conditions, the changes of the most beneficial organization, on the optimum leaf area, for production of dry matter, in the growth period, have been investigated.

The methods used in the analysis are as follows: at first, replacing the

variables, except for the radiation, in the above-mentioned "general formula of dry matter production" with measured values in every stage, and then changing radiation ( $L$ ) as a variable. Results obtained by means of such procedures are illustrated in Fig. 32 (1)–(5), which illustrate, respectively the relations at the

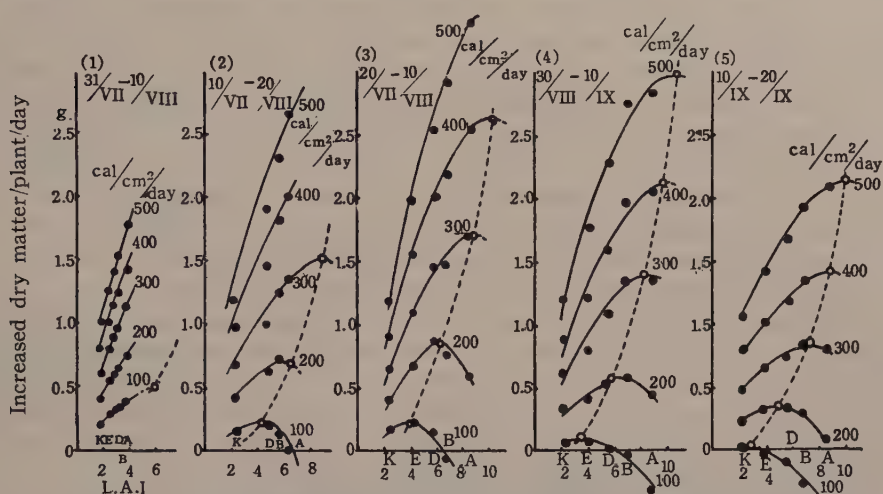


Fig. 32. Relationship between LAI and production of dry matter under various light conditions.

maximum tillering stage (31/VII–10/VIII), young panicle developing (10/VIII–20/VIII), from booting to heading (20/VIII–31/VIII), from heading to early ripening (31/VIII–10/IX), vigorously ripening (10/IX–20/IX). On the abscissa is shown the produced dry matter according to various climatic conditions in each period and on the ordinate the mean leaf area index of each plot during the same period. Mean temperatures during each period are postulated to be 25°C at the (1), (2) and (3) stages, and 24°C at the (4) and (5) stages.

As shown in figure (1), in the (1) stage increase in produced dry matter is associated with increase in mean LAI under all light conditions. Under 100 cal. radiation, the LAI at which production of dry matter attains its maximum is ca. 5.8, and can be deduced theoretically that when leaf area increases beyond this optimum point, the produced dry matter will reduce again.

In figure (2), it can be seen that under unfavourable conditions of 100 cal. radiation, up to a certain limit of LAI ca. 4.0 dry matter production increases with increase in mean LAI, through beyond this limit the production decreases again. Moreover, in these cases, the plots *D* and *E* which are supplied with lesser amount of nitrogen fertilizer rather exceed the plots *A* and *B* which are supplied larger amount of the fertilizer in the production of dry matter. Under 200 cal. radiation, the optimum LAI increases up to ca. 6.0. Under 300 cal. radiation, it attains ca. 8.0. If light conditions become more favourable, increments of dry matter production are proportional to increments of LAI.

The relations in the stage from booting to heading are shown in figure (3). In general at this stage total leaf area attains its maximum and respiratory

ability becomes most vigorous. Under unfavourable light conditions (100 cal.), a optimum LAI is ca. 3.7, if total leaf area increases beyond this point, dry matter production decreases. Under 200 cal. radiation, the optimum LAI increases further, and reaches ca. 5.9. If the light conditions become favourable further, the optimum LAI grows larger in accordance with light conditions. Under 400 cal. radiation, increments of dry matter production are directly proportional to increments of LAI till it reaches ca. 6.0, and under 500 cal. radiation it is ca. 8.0. In both cases if LAI increased beyond these limits, the relationship between LAI and produced dry matter becomes curved.

In figure (4) are illustrated the relations in the stage from heading to early ripening. In this stage field photosynthetic ability declines slightly and total leaf area also decreases a little, but respiratory ability is as vigorous as before. Under weak light conditions (100 cal.), optimum leaf area falls down considerably, and attains ca. 3.2. When total leaf area increases beyond 5.5, dry matter production becomes negative. In other words, in these stages under weak light conditions, rice community possessing larger leaf area can not presumably stand respiratory loss. When the light conditions are 200 and 300 cal., the optimum leaf area also increases and attains 5.8 and 7.8 respectively. Furthermore, even under 400–500 cal. radiation, the linear relationship, which is usually observed in earlier stages, between LAI and dry matter production can not be seen in these stages, the relationship is represented by a curved line.

Figure (5) shows the relations obtained in ripening stage. In general, at this stage photosynthetic ability declines considerably, and total leaf area also decreases. Contrary to decrease of leaf area, non-photosynthetic organs increase. Accordingly, the ratio of photosynthetic gain to respiratory loss becomes the least. In such a case, if the light conditions are unfavourable (for example 100 cal.), increment in dry matter does not take place in any plot. In the plots supplied with larger amounts of nitrogen than plot *E*, respiratory loss exceeds photosynthetic gain, and consequently, even decrease in dry matter is followed. Under 200 cal. radiation, the optimum leaf area reaches ca. 4.4, and under 300 cal., it becomes ca. 6.5. Furthermore under 400 and 500 cal. radiation, it grows gradually to ca. 7.8 and 9.2 respectively. Even in such favourable conditions, no linear relationship between LAI and dry matter production can be seen, and it becomes curved. These relationships can be explained by Fig. 33. Field photosynthetic ability does not increase proportionally with the increase of leaf area due to depression of light receiving coefficient, because mutual shading parallels the increment of leaf area. On the other hand, the community possessing a larger leaf area rather suffers higher respiratory loss. Consequently, produced dry matter as a resultant of photosynthetic gain and respiratory loss reaches a plateau, after when it does not increase so much.

In several curves of Fig. 34 are collectively illustrated the relationships between radiation and optimum leaf areas for each stage with varying light intensities. As shown in the figure, optimum leaf area becomes smaller with advancement of growth stages. These reasons, as explained already, are based on the facts that respiratory loss is concerned considerably with dry matter production, which is the resultant of photosynthetic gain and respiratory loss,



and that the importance of respiration becomes greater with advancement of growth stages. Furthermore, in early stages, leaf blade contains large amount of nitrogen, and possesses high photosynthetic ability, and since non-photosyn-

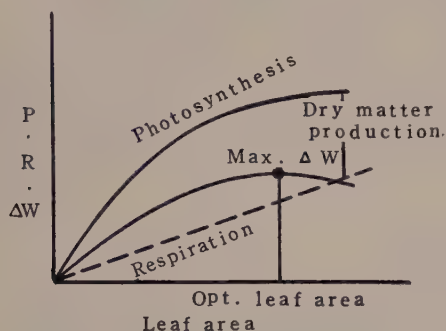


Fig. 33. Relationship between leaf-area and dry matter production.

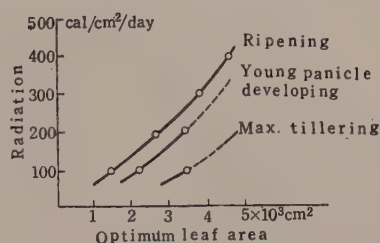


Fig. 34. Relationship between radiation and optimum leaf area.

thetic organs are smaller, the loads due to respiratory loss are lighter. With advancement of growth stages, photosynthetic parts increases progressively, especially during the stage from booting to ripening a large amount of nitrogen contained in leaf blade begins to move to the panicle. As a result, non-photosynthetic parts collect a large amount of nitrogen, and consume one-sidedly substrates by respiration without doing photosynthesis. Increase of respiratory loss with advancement of growth stages results in decrease of the optimum leaf area for production of dry matter under a given radiation in accordance with growth stages.

With regards to mean radiation per day during 10 days of July, August and September in Japan, in favourable condition it rises up to ca. 400 cal./cm², on the other hand, in an unfavourable condition it falls down to ca. 100 cal. Usually the mean values remain within 250–300 cal. Here, having postulated 300 cal./cm²/day as a mean radiation during summer, the changes of optimum leaf area for propuction of dry matter at different times of the growth period have been investigated. These relations are illustrated in Fig. 35. Here, it must be taken into consideration that only the relations under 300 cal. picked up from Fig. 34, are shown in this figure. For the purpose of simplifying the comparisons, all values are shown in percentage of maximum value in each stage. From the figure, it can be understood that the optimum leaf area becomes smaller with advancement of growth stages.

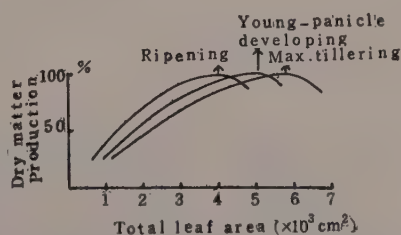


Fig. 35. Changes in optimum leaf-area with advancement of growth.

Under a constant radiation, optimum leaf area changes with advancement of growth stages, as stated above. In actual cases, however, radiation varies considerably. In general, the optimum leaf area depends upon the climatic



conditions. Hence, if the climatic conditions are favourable, optimum leaf area becomes larger as a whole, and if unfavourable it becomes smaller. In other words, the optimum leaf area is a dependent function of environmental factors (especially of radiation). Considering the climatic conditions during the period of rice cultivation in Japan, it may be expected that the optimum leaf area is larger during July and August when the weather is usually fair and smaller during September and October when the weather is rather cloudy. This might be very serious for the rice cultivation, because the optimum leaf area, which has a definite importance to the yield, must be reduced during the ripening period of rice plant.

### SUMMARY

In order to understand the photosynthesis in connection with the yield productivity and to obtain useful information about rice cultivation, the author has attempted a study on the photosynthesis of the rice plant under community conditions, making clear the relationship between photosynthesis and production of matter.

- 1) Rice plants "Norin No. 29" were grown under artificial community conditions with various nitrogen levels, and the changes in the factors constituting field photosynthetic activity of an individual plant in the community were measured.
- 2) On the basis of the obtained results, the determining factors of the field photosynthetic activity were analysed, and the relationships between the changes of the factors and the internal conditions of the plant were investigated.
- 3) Relationships between the factors constituting field photosynthetic activity and dry matter production of the rice plant community were studied, and a general formula of dry matter production was deduced.
- 4) In conclusion the author made clear the significance of leaf area and photosynthetic ability on a leaf area basis in practical rice cultivation.

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## COLCHICINE EFFECT ON POLLEN MOTOR CELLS AND POLLEN GRAINS OF *ZEBRINA PENDULA* SCHNIZL

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AND

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### INTRODUCTION

The use of colchicine as a polyploidizing chemical is well-known. "The penetrability, low toxicity along with the complete recovery through reversibility by the cell are unique qualities of colchicine for doubling the number of chromosomes in plants" [1]. It has been applied successfully through various methods specially to the seeds, seedlings, roots and pollen grains. The importance of polyploidization not only lies in the induction of gigantism in characters, but also in the production of fertile seeds from sterile hybrids.

Though tremendous amount of work has been done in this direction, the effect of colchicine on meiotic cells is yet to be thoroughly investigated [1]. The extent to which the pollen mother cells, with their differentiated cytoplasmic constitution, respond to colchicine action, is not entirely known. There is still much scope of work in this direction.

*Zebrina pendula*, a cultivated species of the family of Commelinaceae is an interesting material for study. The cytology of the species has been thoroughly worked out both by Darlington [2] and Sharma [3]. From the report of the latter author, it has been evident that a high frequency of meiotic irregularities characterizes this species and seed setting has never been noticed in the Indian representatives. Evidently, it has been thought that the meiotic aberrations contribute to the seedlessness of the species.

A scheme of work has been contemplated involving the action of colchicine on meiotic cells of *Zebrina pendula*. The purpose of undertaking the work is twofold. Principally it was desired to find out the extent of the effect of colchicine in inducing polyploidy and its subsequent effects on the pollen mother cells of the species. Secondly, it was thought that this attempt may lead, if possible, to an elimination of meiotic aberrations thus causing a decrease in the sterility. It will be evident from the text that though the sole purpose of the investigations has been covered, i.e. an understanding of its action on meiotic cells has been clearly obtained, yet seedlessness has not been eliminated from the species. Incidentally, it may be mentioned that a modified method of colchicine application on meiotic cells has been worked out during the course of this work, facilitating the operation.

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## MATERIAL AND METHODS

*Material*

*Zebrina pendula* Schnizl. was selected for the present course of our study. It is an ideal material for cytological studies, in which meiotic behaviour shows the evidence of structural hybridity manifested by the formation of rings and chains and by high meiotic irregularities during the divisional stages. A large number of pollen mother cells during anaphase shows lagging of both bivalents and univalents, unequal separation of chromosomes in two poles in first and second anaphase, non-disjunction, and inversion bridges with acentric fragments. The presence of different haploid numbers within the pollen grains resulting out of meiotic irregularities was shown in this laboratory [4] earlier. The plants were grown within the college compound and experiments were carried out under field conditions.

*Methods*

For inducing polyploidy, colchicine in different concentrations, was used and different methods were tried for its application.

I. *Injection*: Different concentrations of colchicine (1 %, 0.5 % and 0.25 % solutions in distilled water) were injected through a fine needle at the base of the scape by maintaining an overhead pressure of the fluid. However, the process was unsuccessful as the pressure was not enough for penetration and probably the soft tissue clogged the lumen of the needle. No effect was produced and meiosis showed normal behaviour even after repeated trials.

II. *Plugging*: Pieces of absorbent cotton soaked in different concentrations of colchicine were applied over the young scapes. The cotton was kept moist by frequent application of the fluid. After treating the scapes with colchicine they were thoroughly washed in distilled water and effect was observed after 7, 14 and 21 days. Flowers opened normally but anthers treated in 1 % solution appeared dry. No mother cells could be seen within the buds and no effect was observed in pollen grains. However, the scapes treated with 0.5 % and 0.25 % showed no effect and normal meiotic divisions could be seen.

III. *Soaking*: Since the two previous techniques were unsuccessful, a new method was adopted, which became very successful. To avoid greater evaporating surface specimen tubes of  $1/2'' \times 2.5''$  dimension were taken and half filled with colchicine solution of different concentrations, viz. 1 %, 0.5 % and 0.25 %. The tips of the two horizontal bracts enclosing the inflorescence proper were trimmed off and other leaves near the scapes were also removed. As the stems were soft and bent easily, the entire scape was introduced in an inverted position within the tube containing the solution. Only the inflorescence was allowed to dip within the fluid. The mouth of the tube was plugged with a moist cotton, taking care that the twig was not injured. After treatment for a definite period, the scape was thoroughly washed in distilled water to remove the superficial adhering alkaloid and to minimize the toxic action of colchicine. The tube was fixed in a vertical position and the height was adjusted according to necessity. The experiments were carried out in different concentrations and for different intervals of time. The observations may be summarized in the following table.



## OBSERVATIONS

TABLE I.

Concentration	Hours of treatment	No. of days	Observation
1%	1 hr.	7	The young buds within the scape dried, anthers in the mature buds deformed.
1%	1 hr.	14	Anthers dried, few flowers opened, no differentiation of the pollen mother cells, appearing black under the microscope.
1%	1 hr.	21	—do—
1%	2 hrs.	7	Buds withered, the inflorescence not properly developed.
0.25%	1 hr.	7	Buds normally developed, pollen mother cells and pollen grains showing no effect, flowers opening normally.
0.25%	1 hr.	14	Buds normally formed, pollens slightly shrivelled, divisional pollen mother cells could not be seen, younger ones with enlarged nuclei.
0.25%	1 hr.	21	Anthers deformed, appearing empty and black under the microscope, pollen grains and pollen mother cells could not be observed.
0.25%	2 hrs.	7	Flowers opened normally, anthers dried, micronuclei in the tapetal cells observed, pollen mother cells undeveloped appearing completely dry.
0.25%	2 hrs.	14	Pollen colourless, anthers in the dividing buds dried, few micronuclei in the body cell.
0.25%	2 hrs.	21	Divisional stages could not be observed, pollen mother cells within young buds enlarged with large nuclei, mature anthers dried with colourless pollens.
0.5%	1 hr.	7	Some of the flowers opened, with dried anthers, pollen mother cells within divisional buds dried, appearing blackish under the microscope.
0.5%	1 hr.	14	The whole inflorescence extremely swollen, buds both young and mature nearly globose, colour of the buds changed to greenish white with slight pink tips; polyploidy observed in divisional stages also. Immature pollen mother cells in young buds highly enlarged with large nuclei, dividing pollen mother cells highly enlarged with numerous bivalents (Photomicrographs 1 & 2). Small and large pollen mother cells found to be present within same anther (Ph. 4), number of bivalents increased even within smaller pollen mother cells. Cytoplasm full of refractory, globular, oily matter. Chromosomes

(Continued)

TABLE I.

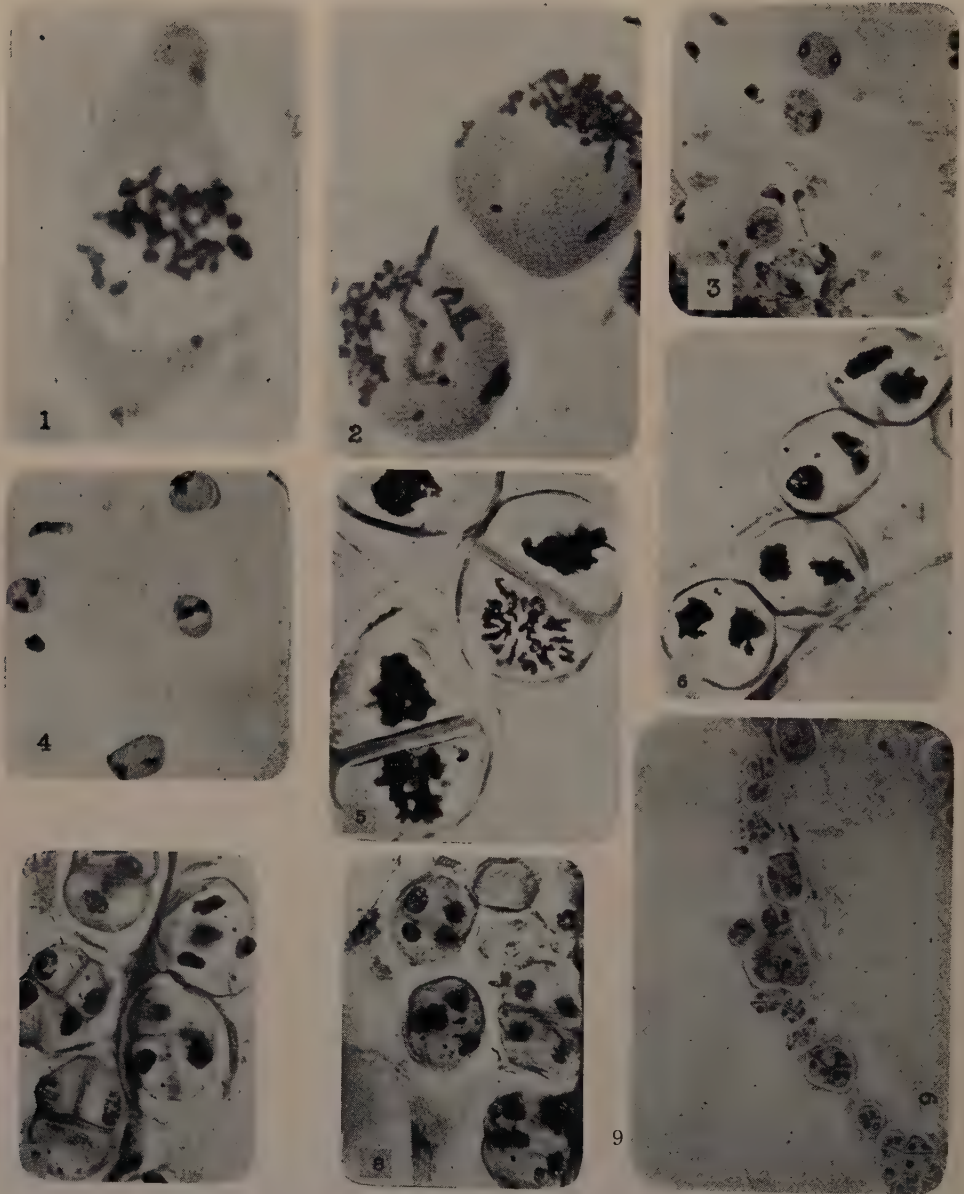
(Continued)

Concentration	Hours of treatment	No. of days	Observation
0.5%	1 hr.	14	<p>brightly stained and more or less clumped, individuality not completely lost.</p> <p>Second meiotic metaphase and late anaphase chromosomes showing a peculiar astral configuration (Ph. 3). Twenty-four chromosomes in second metaphase polar view could be counted (Ph. 5). Large number of lagging chromosomes in anaphase of both first and second meiotic divisions noted. Early separation, non-disjunction highly prevalent (Phs. 3-8).</p> <p>Later stages of development showing successive division of the pollen mother cells, resulting into the formation of polysporous state of varying size (Phs. 7-10). Extra nuclear chromosomes frequently observed with the result of abnormal divisions.</p> <p>Pollen grains of different sizes noted, some extremely enlarged (Ph. 12) with no significant enlargement of the nuclei, small pollens occurring side by side showed no polyploid number. In one of the pollens 12 haploid chromosomes could be counted. In enlarged nuclei no polyploid number could be seen.</p> <p>Extra nuclear chromatin matter found to be deposited in the periphery of the pollen grains (Ph. 11).</p>
0.5%	1 hr.	21	<p>Some of the mature buds dried, others swollen, divisional stages not found, pollen mother cells in the swollen buds with enlarged nuclei; pollens in mature buds deformed and colourless.</p>
0.5%	2 hrs.	21	<p>The anthers appeared dry, pollens deformed and colourless, buds withering.</p>

## DISCUSSION

Application of colchicine on young inflorescence presented a lot of difficulties and as pointed out in the text, a new method had to be developed, which has been quite successful. Of all the concentrations tried so far, 0.5% solution yielded best results with *Zebrina pendula*. In low concentrations, no appreciable effect could be recorded with low period of treatment whereas under prolonged treatment, withering and lethality of the tissue ensued. This indicates that irrespective of the concentration, continued application of the chemical causes a drastic metabolic disorder, finally resulting in lethality.

After treatment for one hour in 0.5% solution the tissue showed signs of



Phs. 1-9. Colchicine effect on PMCs of *Zebrina pendula*. 1 and 2, showing colchicine induced polyploid, enlarged PMCs.  $\times 1100$ - $\times 1000$  approx. 3 and 4, asteroid M II and abnormal divisional stages in colchicine induced polyploid PMCs  $\times 200$  approx. 5, colchicine induced second meiotic metaphase showing high number of chromosomes in one pole.  $\times 900$  approx. 6 and 7, abnormal meiotic stages occurring at high frequency in colchicine induced polyploid PMCs.  $\times 720$  approx. 8, peculiar successive division together with laggings.  $\times 660$  approx. 9, showing size difference, abnormalities and peculiar successive division in later stages of development.  $1 \times 200$  approx.



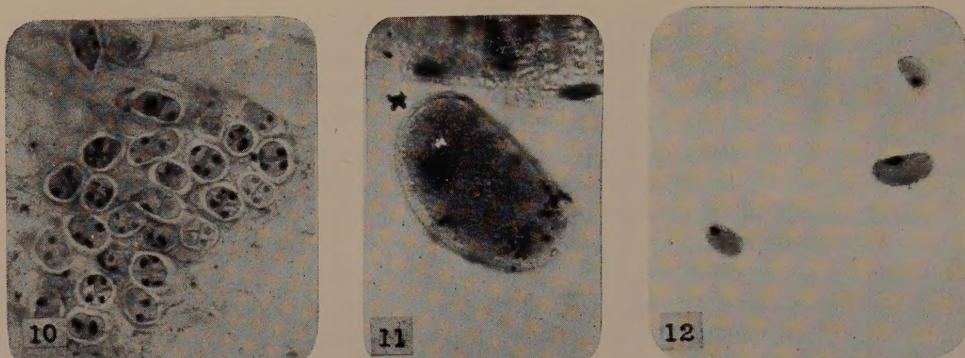
polyploidy after fourteen days of growth. As colchicine was applied to young inflorescence, it can be inferred that the tissue showing division after fourteen days, initially received the chemical at an extremely young condition. Buds maturing after seven days on the other hand did not show any sign of polyploidy and non-dividing and shrivelled pollen mother cells were obtained. These facts may be taken to imply that colchicine application in this species is only effective if applied on extremely immature cells, i.e. cells lying in a very early phase of development.

The manifestation of effect in a bud after fourteen days further suggests that colchicine retards to an appreciable extent the normal rate of growth of the young floral buds. Untreated inflorescence of this species normally matures within three to four days after just the emergence of the inflorescence and all the flowers open completely by six to seven days at the latest. In colchicine treated materials on the other hand, buds appear even after fourteen days indicating thereby that the growth rate is being retarded to a considerable degree. It will be worthwhile to find out the extent to which this retardation of growth of the floral buds can be compared with the results obtained with the other tissues as well.

A point of interest regarding colchicine action on pollen mother cells involves the nature of chromosome separation during anaphase. It has been found that heavy irregularities characterize the behaviour of the chromosomes of pollen mother cells. Irregularities include lagging, early separation, extrusion of chromosomes from the spindle, etc. Giant size of the pollen mother cells and of the pollen grains have also been noted. In none of the cases regular division of the polyploid cells is present. This is in strong contrast to the effect of colchicine on root cells, where the roots recover within one or two days after treatment and regular division of the polyploid nuclei has been found. Evidently, this differential effect is due to the compact nature of the cells of the roots in contrast to the loosely arranged mother cells within the anther cavity. The compact nature possibly allows an easy recovery as in such cases the effect exerted on the tissue is distributed by cell to cell diffusion and the injurious effect, as such, is gradually coped with. On the other hand, in pollen mother cells, the mother cells being individual units, receive the full effect of the chemical, and such, regular division is scarcely obtained. Colchicine action, being strong in its impact, does not allow a normal behaviour of the pollen mother cells in which, under natural conditions, certain metabolic equilibrium is maintained facilitating the complex process of chromosome pairing, reduplication and separation.

Another feature worthnoting is the successive mitotic division after meiosis in the pollen mother cells after reduction. This is possibly a means through which the cells try to revert back to the normal condition and to be immunized against the injurious effect of the chemical. This behaviour has been noted in roots when the roots are kept immersed in colchicine solution for a longtime [4]. Evidently, the retention of colchicine within the tissue gradually compels the cell to undergo a natural reaction resulting in immunity in roots. In pollen mother cells on the other hand, this behaviour is observed on the fourteenth





Phs. 10-12. *Zebrina pendula*, colchicine induced PMCs. 10, showing size difference, abnormalities and peculiar successive division.  $\times 200$  approx. 11, colchicine induced enlarged pollen grains showing extrusion of chromatin matter.  $\times 720$  approx. 12, showing size difference of the pollen grains after polyploidization through colchicine.  $\times 150$  approx.

day after treatment. This is a clear indication of the fact that colchicine effect remains accumulated in the pollen mother cells, allowing such successive mitotic division. The possible cause is the individual nature of the pollen mother cells, which are not arranged in a compact way, and so the accumulated colchicine cannot have a proper readjustment within the tissue as a whole, through cell to cell diffusion.

### SUMMARY

The action of colchicine on the pollen mother cells of *Zebrina pendula* Schnizl. has been observed. Polyploidy has been successfully induced in the floral scape of *Zebrina pendula* through dipping the whole inflorescence in 0.5 % colchicine solution for one hour with the aid of an improvised method. A delayed response after fourteen days suggests that early embryonic stages are more susceptible to the action of colchicine in this particular species. In higher concentrations the effect is toxic while in lower concentrations, polyploidy was not induced.

Both external and internal manifestations of polyploidy in the whole inflorescence have been noted. Buds have been enlarged with fading of normal colour. Pollen mother cells in early and divisional stages appear highly enlarged with large nuclei in the former and numerous bivalents in the latter. In first and second meiotic divisions high irregularities have been noted, manifested by lagging of chromosomes, early separation, non-disfunction and extraspindle-chromosomes. A peculiar successive division of pollen mother cells has been observed. Pollen grains have been found to be highly enlarged but increase in the number of chromosomes has not been recorded. Extra-nuclear chromatin matters have been found within the pollen grains.

The evidences obtained here show that the manifestations of colchicine effect are different in pollen mother cells as compared to that of roots worked out by different authors. This has been suggested as due to the loose arrangement of the former. The pollen mother cells as such, behave as independent units and do not allow cell to cell diffusion, which otherwise in roots causes the tissue to

recover from the injurious effect of the cell. All the differential effects have been shown to be ultimately due to this characteristic set up of pollen mother cells.

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